GENE EXPRESSION AND FATIGUE IN PUERTO RICAN MEN

BY



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## Abstract

**Background:** Prostate cancer is the most frequently diagnosed cancer in men (40.6% in 2010) in Puerto Rico and external beam radiation therapy (EBRT) is the preferred form of treatment for one third of newly diagnosed prostate cancer patients in Puerto Rico. Puerto Ricans often experience ethnic disparities in cancer treatments and in their symptom experience.

**Purpose:** This study will: (a) describe the trajectory of fatigue among Hispanic Puerto Rican men over the course of receiving EBRT for non-metastatic prostate cancer and compare these findings with historical data of fatigue symptoms of Caucasian men with prostate cancer during EBRT; (b) assess gene expression changes from baseline to midpoint of EBRT using microarray technology; and (c) determine the association between changes in genes expression and changes in fatigue score from baseline to midpoint of EBRT using an unbiased, hypothesis-generating approach.

**Methods:** As a prospective exploratory and comparative design study, fatigue was measured using the Functional Assessment of Cancer-Therapy –fatigue from 26 Hispanic Puerto Rican men who were newly diagnosed with non-metastatic prostate cancer at three time points (baseline [prior to EBRT], midpoint [days 19-21], and end of EBRT [days 38-42]). Whole-blood samples also were collected at baseline and at midpoint of EBRT to explore the differential expression of genes using microarray. Functional networks of the differentially expressed genes were examined. Descriptive data were analyzed using t-test, Wilcoxon, and Friedman test for repeated measures. Gene expression data were analyzed using the *LIMMA* package in R and the functional network analysis was conducted using Ingenuity Pathway analysis.

**Findings:** Subjects were ages 52-81 with fatigue scores that were unchanged during EBRT (baseline= 42.38, *SD*= 9.34; mid-point=42.11, *SD*= 8.93, endpoint= 43.04, *SD*= 8.62);

Friedman's test: $\chi^2$1.20[*df*, 2], $p$=.55). Three hundred seventy-three genes (130 up regulated and 243 down regulated) were differentially expressed from baseline to mid-point of EBRT (FDR<0.01). The top distinct canonical pathways of the differentially expressed probesets (p< 0.0001) were: Phospholipase C Signaling, Role of NFAT in Regulation of the Immune Response, and Gαq Signaling.

**Conclusions:** There were no changes in fatigue scores among Puerto Rican men during EBRT for prostate cancer. However, differentially expressed genes during EBRT suggest activation of immune response, which is a mechanism proposed to explain cancer-related fatigue. Further investigation is warranted to explain the disparity in fatigue symptoms reporting of Puerto Rican men from other ethnicities receiving the same treatment.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| • Androgen Deprivation Therapy | ADT |
| • Body Mass Index | BMI |
| • Cancer Related Fatigue | CRF |
| • deoxyribonucleic acid | DNA |
| • External Beam Radiation Therapy | EBRT |
| • Functional Assessment of Cancer Therapy – Fatigue subscale | FACT-T |
| • Gray | Gy |
| • Hamilton Depression Rating Scale | HDRS |
| • Health-Related Quality of Life | HRQOL |
| • International Physical Activity Questionnaire-Short Form | IPAQ-SF |
| • messenger RNA | mRNA |
| • mitochondria DNA | mtDNA |
| • Principal investigator | PI |
| • Patient-Reported Outcome Measures Information System for Sleep Disturbance - short form | PROMIS-sleep disturbance-SF |
| • Radiation Therapy | RT |
| • Reactive Oxygen Species | ROS |
| • ribonucleic acid | RNA |

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# CHAPTER I

# INTRODUCTION

## Background

Prostate cancer is the most frequently diagnosed cancer (40.6% in 2010) in Puerto Rico and the leading cause of cancer death among Puerto Rican men.[1] Deaths from prostate cancer in Puerto Rico are higher than in all racial groups in the U.S.[2] Hispanic Puerto Rican men are more likely to experience disparities in cancer treatments such as later stage diagnosis requiring more intense treatments, worse symptoms and poorer health-related quality of life (HRQOL) when compared to Caucasians in the U.S.[3-7] Many newly diagnosed prostate cancer patients in Puerto Rico are not willing to undergo prostatectomy probably due to fears about possible side effects (e.g., chronic urinary incontinence and sexual dysfunction), making external beam radiation therapy (EBRT) a more popular option in the management of non-metastatic prostate cancer in Puerto Rico.[1,4]

While localized treatments like EBRT have led to higher cure rates,[9] up to 71% of prostate cancer patients complain of fatigue during EBRT.[10-11] This fatigue has been associated with alterations in employment, increased hospitalizations, non-compliance with treatment, and need for dose adjustment or interruption of treatments.[13] Cancer-related fatigue (CRF) is a multidimensional symptom also influenced by psychological and physiological factors. For example, a systematic review [8]showed that depression was significantly correlated with fatigue (95% CI, 0.54 to 0.58) across the 51 studies. Sleep disturbance also has been found to be positively correlated with fatigue and to be a significant predictor of fatigue.[12] Similarly, increased general fatigue was found to be significantly associated with reduced vigorous physical activity and physical activity has been shown to have some benefit in the management of fatigue.[13] Nonetheless, recent studies suggest intrinsic biological vulnerabilities may contribute to, or explain the higher prevalence of cancer therapy-related symptoms in Hispanics than among Caucasians.[7,14] The etiology and mechanism underlying this disparity remain elusive.

*In vivo* studies suggest that differential expression of genes activating several physiological pathways may explain the mechanisms in CRF.[15-19] Recent research using microarray technology to identify possible biomarkers of CRF among Caucasian men receiving EBRT for non-metastatic prostate cancer found 11 mitochondrial-related differentially expressed genes, eight of which showed significant associations with changes in fatigue scores.[16] Much remains to be investigated, including whether physiological evidence can explain the variability of fatigue responses to EBRT between ethnic groups, especially in the Hispanic Puerto Rican male population.

## Purpose of Study

The proposed study explored changes in self-reported fatigue and gene expression over the course of EBRT. In order to capture the initial inflammatory response of EBRT that peaks at midpoint (day 21),[15-19] we focused on determining the changes in expression of genes from whole blood samples that were collected at baseline and midpoint of EBRT only. Functional networks of the differentially expressed genes were examined using Ingenuity Pathway Analysis to determine pathways that may explain the possible physiological mechanisms that influence fatigue intensification during EBRT in this population.

## Fatigue during EBRT

Cancer-related Fatigue (CRF) is one of the most unpleasant and distressing symptoms experienced during treatment.[10,13,15] While CRF during EBRT appears unrelated to age, cancer stage, radiation dose or fraction, it is frequently associated with racial and ethnic differences, and reduced health related quality of life (HRQOL) and physical functioning.[5-6,10-12] Further, it consistently has been reported that the occurrence and severity of CRF in Hispanics, of which Puerto Rican men are a sub-group, is higher than in Caucasians.[14,20] As an example, Gonzalez and colleague's preliminary study using the Therapy Related Symptom Checklist showed that Puerto Rican men with prostate cancer ($N$=13) have a high prevalence (80%) of fatigue at midpoint of EBRT.[25] There is increasing recognition that variability in the symptom experience of fatigue may be explained by biological causal pathways.[21-24] Recently, Saligan and colleagues found a significant association between fatigue intensification during EBRT of Caucasian men with non-metastatic prostate cancer and up regulation of *IFI27* (expression value = 0.774, $p < 0.0001$), a gene known to induce apoptosis that is highly induced by IFN-α/β, both cytokines that alter immune response.[17] Not only is there a need for more exploration examining cultural differences in this area, but identifying whether or not there is a difference in physiologic profiles associated with fatigue intensification during EBRT among ethnic groups will contribute to symptom science. Moreover, the Puerto Rico Comprehensive Cancer Control Plan (2008-2013)[2] and the 2013 Oncology Nursing Society Research Agenda[26] have called for more investigations on the incidence and etiology of CRF. There currently is no optimal pharmacologic therapy and little molecular evidence to guide the development of effective therapies for the management of CRF.

## Biological basis for EBRT-related Fatigue

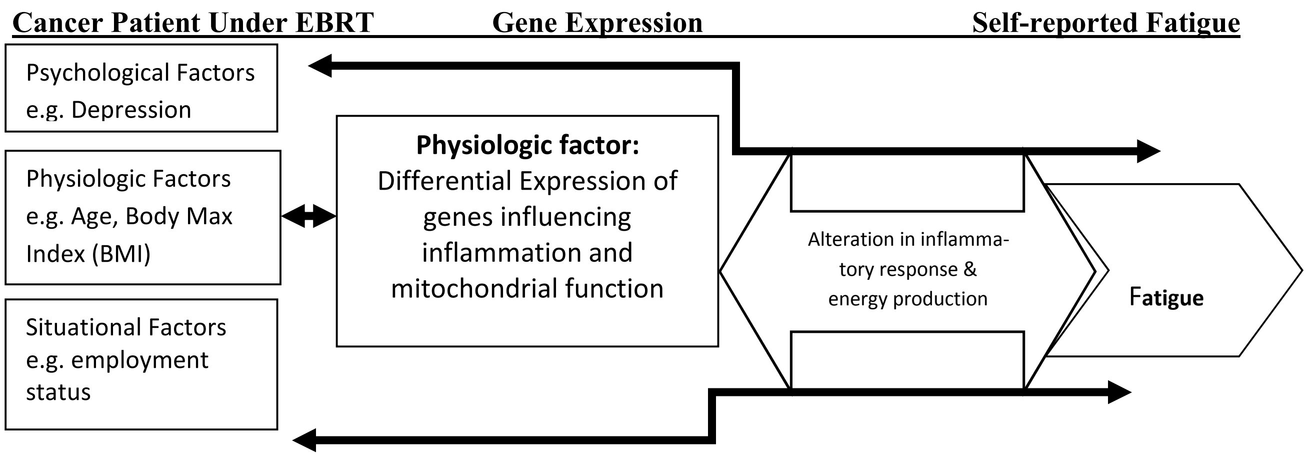
Gene expression levels are known to change in response to genotoxic stress, such as ionizing radiation, including those involved in cell cycle control or deoxyribonucleic acid (DNA) repair, as well as transcription factors, growth factors and proteases.[27] Furthermore, EBRT affects many biological networks, including those related to immune response and mitochondrial function.[16-19] Both of these mechanisms can contribute to the development of fatigue that compromises the quality of life of cancer survivors. Therefore, a plausible etiology of EBRT-related fatigue[28] is based on the fact that in addition to reducing cell survival, ionizing radiation has been shown to simultaneously induce accelerated biological aging leading to cellular senescence containing dysfunctional mitochondria. Dysfunctional mitochondria can impair energy conversion and increase the production of reactive oxygen species (ROS) and reactive nitrogen species. These induce detrimental cellular damage to irradiated cells and cells of surrounding normal tissues, causing short and long term bystander effects through cytokine stimulation to respond to tissue damage. Genomic changes could mediate some of the effects of ionizing radiation.[16-19] Therefore, much interest in how variations in gene expression patterns can play a part in the genetic differences among patients that culminate in dissimilarity in the experience of fatigue has emerged. Saligan's group further observed a significant correlation between changes in fatigue and the upregulation of *ApoE*[18] and α-synuclein during EBRT[17], providing initial evidence of the role of inflammation, mitochondrial dysfunction and neuro-metabolism behind fatigue intensification during EBRT of Caucasian men with non-metastatic prostate cancer.

The knowledge base of EBRT-related fatigue is increasing; yet, limited attention has been given to provide physiological evidence that can support or explain the disparity in the symptom experience of fatigue between ethnic groups, especially in the Hispanic Puerto Rican population. This disparity in symptom experience influences treatment outcomes and clinical management of symptoms.[5,7] Further investigations are needed.

## Conceptual Framework

The proposed study is informed by an innovative conceptual framework that draws on the Theory of Unpleasant Symptoms (TUS).[29-30] The TUS includes three major concepts: symptoms, influencing factors, and consequences of the symptom experience.[29-30] The TUS posits that interactions among the influencing factors (physiological, psychological, and situational) affect predispositions to manifest unpleasant symptoms.

**Figure 1. Model of Gene Expression and Cancer-Related Fatigue**




In this model (see Figure 1), an interrelationship exists between psychological, physiological, and situational factors and the fatigue experience during EBRT. A variety of physiological factors have been postulated to be associated with RT-associated fatigue. Radiation-related damage induces cellular responses manifested by differential expression of genes influencing activities of physiologic pathways including mitochondrial function, inflammation, DNA damage processing, inhibition of signal transduction, mutations, cell-cycle arrest, genomic instability, carcinogenesis, and cell death.[28] Changes in levels of gene expression are posited as interacting with, and affecting, the person's predisposition to develop fatigue through alterations in the individual's response to inflammation and oxidative stress. Evidence of CRF associated with age, body mass index (BMI) > 25 kg/m$^2$, sleep disturbance, and low energy expenditures, have been reported and CRF impairment may be partially induced or exacerbated by psychological factors such as depression.[12,31-34] Age, BMI, sleep disturbance, energy expenditure, and depression will be measured in this study. Other physiological factors (e.g. disease/treatment characteristics) and situational factors (e.g. employment status) will be used to describe the sample since such factors have not been associated with CRF.[35-36] Changes in gene expression and self-reported CRF will be the main variables to be investigated.

## Study Aims

*The proposed study specific aims are:*

1: To describe the trajectory of fatigue among Hispanic Puerto Rican men over the course of receiving EBRT for non-metastatic prostate cancer and compare these findings with historical data of fatigue symptoms of Caucasian men with prostate cancer during EBRT.

2: To assess gene expression changes from baseline to midpoint of EBRT among Hispanic Puerto Rican men receiving EBRT for non-metastatic prostate cancer.

3: To determine the association between changes in genes expression with changes in fatigue scores from baseline to midpoint of EBRT in Hispanic Puerto Rican men with non-metastatic PC.

## Design Overview

In this study, the clinical fatigue experienced by 26 Hispanic Puerto Rican men over the course of EBRT was described. Then, an unbiased hypothesis-generating approach using a microarray platform was conducted to explore the differential expression of genes from peripheral whole blood RNA collected from Hispanic Puerto Rican men at baseline and at midpoint of EBRT. Because of resource limitations and in order to capture the initial inflammatory response of EBRT which peaks at midpoint (day 21), only levels of the differentially expressed genes at baseline and at midpoint of EBRT were determined. Functional networks of the differentially expressed genes were examined using Ingenuity Pathway Analysis to determine pathways that may explain the possible physiological mechanisms that influence fatigue intensification during EBRT in this population.

# CHAPTER II

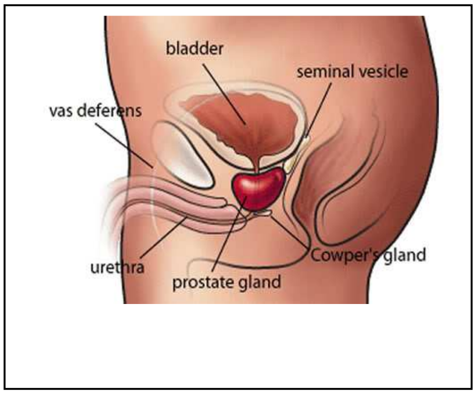
## Review of the Literature

The review of the literature begins with a detailed discussion of prostate cancer including pathophysiology, prevalence and mortality rates, and treatment options. A general background on Puerto Rico (P.R.) including cancer mortality and morbidity rates as well as cancer disparities is presented next. This is followed by a discussion of the concept of the symptom experience of Cancer Related Fatigue (CRF), including the pathophysiology and genetic basis for External Beam Radiation Therapy (EBRT)-associated fatigue, gene expression and prostate cancer therapeutics. The Theory of Unpleasant Symptoms (TUS) will guide the consideration of variables discussed that may influence the person's ability to perceive CRF symptoms. Finally, biological vulnerability to disparity in the experience of symptoms will be discussed.

## Prostate Cancer

The prostate is a gland that is part of the male reproductive system. A healthy adult prostate measures approximately 4 x 2 x 3 centimeters and weighs about 20–25 grams (the size of a "large walnut"); size is usually maintained constant throughout early adulthood and the middle years.[37] Figure 2 shows the location of the prostate that is beneath the bladder, surrounding the upper part of the urethra, and in front of the rectum. The main function of the prostate is to produce the seminal fluid, a white substance that nourishes and transports the sperm.

**Figure 2. Location of the Prostate**




Source: http://photobucket.com/images/prostate%20cancer#/images/prostate%20cancer?page=1&_suid=1390846511045063268358055 3763

In order to keep the prostate healthy and functioning properly gland cells grow, divide, and produce more cells as needed; however, if this process goes wrong, gland cells become abnormal and form more cells in an uncontrolled manner.[38] These extra cells form a tumor (mass of tissue) that can be benign (not cancerous) or malignant (cancerous).[38] Therefore, prostate cancer is a disease in which malignant (cancer) cells divide without control or order in the tissues of the prostate gland. Prostate cancer is considered an adenocarcinoma.[39] Most common symptoms of prostate cancer are dysuria, incontinence, weight loss, and weakness. While the exact etiology of prostate cancer is unknown, specific genes (proto-oncogenes and oncogenes) have been implicated in the "transformation" of cells to a state of hyper-proliferation (cancer), which disrupts the cooperation among cells of an organ or tissue.[40] In addition, according to the National Cancer Institute (NCI, n.d.), age (with nearly 63 percent of prostate cancer cases occurring in men over age of 65), race (more common in African American men than in any other group of men in the U.S.), and family history of prostate cancer can affect the risk of developing prostate cancer. Further, high-fat diets also may increase risk (eating fats raises the amount of testosterone in the body, and testosterone speeds the growth of prostate cancer).[39] Current diagnostic criteria for prostate cancer are based on findings from a physical examination, laboratory test and tissue examination.

**Prostate Cancer prevalence and mortality**

Prostate cancer is a curable disease but it is still one of the most prevalent malignancies worldwide.[41] For example, in the United States (U.S.) prostate cancer is the most frequent cancer (after skin cancer) among men; it is estimated that about 238,590 new cases will be diagnosed in 2013.[41] One out of six men will be diagnosed with prostate cancer in their lifetime and more than two million men are living with prostate cancer. The five-year survival rate of non-metastatic prostate cancer approaches 100%; however, for metastatic diseases it is less than 29%.[41] In fact, prostate cancer is the second leading cause of cancer death in U.S. men. Approximately 29,720 deaths among U.S. men in 2013 were caused by prostate cancer and about 1 in 36 men will die of prostate cancer in their lifetime. Prostate cancer also is the most common male cancer in P.R. with 8,510 new cases diagnosed during the period of 2004-2006.[2]

**Treatment Options for Non-metastatic Prostate Cancer**

Current treatment for non-metastatic prostate cancer is based on the National Comprehensive Cancer Network guidelines.[42] Non-metastatic prostate cancer treatment consists of one or a combination of the following: active surveillance, surgery, hormonal therapy, and radiation therapy. According to the National Comprehensive Cancer Network (2012),[42] active surveillance involves actively monitoring the course of the disease to be able to intervene if cancer progresses, and is commonly appropriate for men with very low risk cancers (e.g., older men with low-grade tumors and low Prostate Specific Antigen (PSA) readings) or for patients with short life expectancy (< 10 years). Advantages of active surveillance mentioned in the literature are: avoidance of common side effects of unnecessary cancer treatments, maintenance of good health related quality of life (HRQOL), and reducing risk of unnecessary cancer treatments of small or indolent cancers. However, limitations include: (a) the need for periodic biopsies, (b) chance of missed opportunity for cure, (c) risk of progression and/or metastases then requiring more aggressive treatments, (d) experiencing worse symptoms, and (e) feelings of anxiety and uncertainty about the natural history of prostate cancer. If active surveillance is selected, Digital Rectal Examination and PSA's every three to six months are recommended, as are repeated prostate biopsy within six month of diagnoses if Digital Rectal Examination changes or PSA increases.

Surgery also is recommended to treat clinically non-metastatic prostate cancer. An appropriate and most common surgical option for non-metastatic prostate cancer is the radical prostatectomy in which the cancerous prostate is removed from the body. It is a recommended treatment for patients in which the prostate completely can be excised surgically, with life expectancy > 10 years, and with no serious co-morbid conditions that could contraindicate the surgery. Overall, it is a highly favorable treatment for younger men with early stage disease, although it also may be performed in advanced stages of the disease for symptom relief. It can be performed via open, laparoscopic or robotic technique. Frequently reported side effects are: blood loss, urinary incontinence, loss of erection, and anastomotic stricture.

Additionally, androgen deprivation therapy (ADT), or hormonal therapy, is prescribed to reduce the amount of testosterone that prostate cancer needs to grow. It is recommended for prostate cancer patients with intermediate or high risk of recurrence or local metastasis.[42] ADT consists of once-a-month intramuscular injection of luteinizing hormone-releasing hormone agonist (e.g., leuprolide, goserelin), with bicalutamine that then has the effect of reducing testosterone.[43] While hormone therapy cannot cure cancer, it has been shown to delay its growth and to provide relief. The use of neoadjuvant ADT highly is recommended to be given before, during and/or after radiation therapy (RT) since studies have shown this improves survival.[44-45]

In addition, ADT given at a high dose of 150mg alone has been shown to delay recurrence of disease, but not to improve survival. Commonly reported side effects of ADT are: osteoporosis, obesity, insulin resistance, alteration in lipids, and increased risk for diabetes, and cardiovascular diseases.[42,46]

Traditional RT for prostate cancer has caused concern among health care providers and patients with respect to targeting normal tissues as well as the prostate and about the development of more treatment-related symptoms. Recent advances in image-based radiation treatment planning and localization led to the development of the external beam radiation therapy (EBRT).[9] The purpose of EBRT is to deliver high-energy rays to tumors to inhibit cell proliferation or to induce apoptotic cell death *in vitro* and inhibit tumor growth *in vivo* by damaging the DNA--the molecule inside cells that carries genetic information.[47] Ionizing radiation kills cells by inducing DNA damage such as base damage, single strand breaks, double-strand breaks, and DNA-inter-strand cross-links.[48] As a result of EBRT, cancer cells whose DNA is damaged beyond repair stop dividing or die and then are eliminated by the body's natural processes. The linear accelerator used for EBRT allows radiation beams to be delivered from any angle and shape to the contour of the tumor. Linear accelerators use electricity to form a stream of fast-moving subatomic particles that create the high-energy radiation used to treat cancer. Overall, it is considered a safe state-of-the-art radiation technology that enables targeting a tumor more accurately with higher doses of radiation, while minimizing damage to healthy tissue and nearby organs and diminishing the risk of side effects typically associated with radiation treatment.

Gray (Gy) is the unit of measure used for radiation doses for cancer treatment; it is a measure of the amount of radiation energy absorbed by one kilogram of human tissue. EBRT requires patients to travel to the hospital or an outpatient facility up to five days a week for six to eight weeks to receive once-daily treatment or fractions. Most non-metastatic prostate cancer patients are prescribed an approximate dose escalating to a total daily dose of 75.6 to 79 Gy of a 3-dimensional conformal and intensity modulated radiation therapy in conventional 36-41 fractions (6 to 8weeks), approximately at 2.0 Gy per day.[42] With respect to outcomes, the American College of Radiology reported 83% of adjusted 5-year rates of no evidence of disease with a dose of 75 to 80 Gy.[9] The occurrence of gastrointestinal or genitourinary toxicities or sexual dysfunction are the most common side effects of EBRT reported.[9]

Another type of RT is brachytherapy that consists of surgical implantation of radioactive pellets inside the prostate. Over time, the pellets radiate the prostate and surrounding tissue, killing the cancer cells. Permanent monotherapy of low-dose rate brachytherapy (145 Gy for 125-Iodine and 125 Gy for 103-Palladium) also is indicated for men with low-risk cancers. Common side effects of brachytherapy reported by patients with a very large or very small prostate are symptoms of bladder outlet obstruction. For patients that have a history of transurethral resection of the prostate, brachytherapy may not be appropriate as implantation may be more difficult and they are at increased risk for the side effect of bladder outlet obstruction. Overall, it has been reported that RT generally creates fewer side effects than surgery.[9,46] For this reason, it is often the preferred treatment for older men.

Finally, even though active surveillance, radical prostatectomy, brachytherapy, and RT historically have been considered treatment options for early-stage prostate cancer patients, the most recent consensus from an expert panel on Radiation Oncology[9] recognized EBRT as the standard of care. Recent studies on outcomes after treatment for non-metastatic prostate cancer have shown no benefit of prostatectomy or brachytherapy over EBRT when assessing freedom from biochemical failure, progression-free survival, and performance functional status.[9,49] As a result, EBRT is one of the most popular treatment choices among non-metastatic prostate cancer patients, including Puerto Ricans (PR's),[1]who are not candidates or are not willing to undergo prostatectomy surgery often due to its well-known symptom occurrence of chronic urinary incontinence and sexual dysfunction.

## Background on Puerto Rico

### General Background

The island of Puerto Rico is the smallest and the most eastern of the Caribbean Greater Antilles. Puerto Rico is a geographically diverse archipelago of 78 municipalities with a total land area of 3,424.56 square miles.[50] The 1940s were significant to Puerto Rico for shifting its economy from agricultural to industrial. Since the establishment in 1952 of the Commonwealth of Puerto Rico as the primary basis of governance, Puerto Rico has been politically and economically linked to the U.S. A Governor in Puerto Rico is the highest political figure elected in Puerto Rico. Island Puerto Ricans do not vote for the president of the U.S. Puerto Rico has its own government with Executive, Legislative, and Judicial branches. A Resident Commissioner represents Puerto Rico in the U.S. House of Representatives having a voice but no vote. As part of the Commonwealth benefits, Puerto Ricans have U.S. citizenship, allowing them to visit, work, and live in the U.S. freely.[50] Puerto Ricans have their own sports teams to represent them during international and worldwide events. In addition, the official language of Puerto Rico is Spanish but English is taught as a second language. Puerto Rico remains essentially a Hispanic country with its own cultural heritage, yet, with certain characteristics due to U.S. influence.[50]

Similar to the education system, Puerto Rico has public, private, and privatized public health care institutions. According to the American Hospital Directory,[51] in 2012 Puerto Rico had 54 hospitals, 8 of them public (including privatized public hospitals) and 46 private hospitals. Puerto Rico also has a Veterans Administration Hospital that provides services to members of the armed forces. The government also covers part of the cost for employee's private insurance. It was estimated in 2008 that 1,500,000 patients had Reforma (Government Health Insurance), 1,500,000 had a commercial plan, 380,000 had Medicare advantage, 200,000 had Medicare alone, and 400,000 were uninsured.[52]

With respect to cancer care, when "Reforma" patients are diagnosed and receive cancer treatment, they qualify for a special coverage that gives them easy access to medications and services without the need for pre-authorizations. Private insurance patients with limited cancer coverage can apply for "Reforma" coverage. The Puerto Rico Medical Center as well as the American Cancer Society, Puerto Rico chapter, provides initial cancer care free of cost to those who are temporarily without insurance while waiting for permanent health insurance plans.

There are two specialized cancer adult hospitals in Puerto Rico. One is located in Centro Medico in the metropolitan area of San Juan and one is in Ponce in the southern part of the island. Both are private hospitals that accept "Reforma" insurance. Also, other general hospitals in Puerto Rico offer oncology services. R.T. as well as chemotherapy services are available in several places all over the island, including at oncologist's private offices. There are three Bone marrow units (2 for pediatrics and 1 for adults) available in Puerto Rico, but these programs only offer autologous transplant. Reasons for that may be: limited availability of donors, lack of trust in the system, delay in referrals, and limited qualified personnel. Puerto Ricans also consider visiting U.S. hospitals for cancer evaluation and treatments. The University of Puerto Rico

Cancer Center planned the construction of a hospital (started in 2014) with clinics and specialized services for all the adult and pediatric oncology population of Puerto Rico.

Clinical oncology research has not been abundant, in part due to the limited number of doctoral programs available in the island to prepare researchers. As an example, the University of Puerto Rico started the first Doctor in Nursing Science Program in Fall 2012. Nonetheless, there is hope on the way for pediatric and adult patients to receive comprehensive oncology cancer care in the University of Puerto Rico Cancer Center as well as the option to participate in clinical trials once the new hospital is completed.

**Demography**

According to the U.S. Census, in 2010, the population of Puerto Rico was 3,979,000, and it is expected to increase to 4,024,000 in 2015.[53] The median age was 38.2 years. Twenty-eight percent of the population was under 20 years and 14.1% was 65 years and older. The population ranges from 434,373 in San Juan, the Capital City, to 1,868 in the island-municipality of Culebra. In Puerto Rico, nearly two-thirds of the population lives in the northeastern half of the island. Twenty-nine percent of Puerto Ricans live in rural areas. The less populated municipalities are located in the mountainous center of the island.[53]

A significant emigration of P.R.'s to the U.S. took place between 1947 and 1957, mainly due to unemployment and economic reasons. An estimated 4.7 million Hispanics of Puerto Rican origin reside in one of the 50 states and the District of Columbia. New York has the largest population of Puerto Ricans, with approximately 1.1 million residing there. Since the 1990's Puerto Ricans also have been immigrating to other states such as Illinois, Texas, Florida, Pennsylvania, New Jersey, and Massachusetts. The U.S. 2010 census reported that Puerto

Ricans are the second largest population of Hispanic origin living in the U.S. accounting for 9.2% of the U.S. Hispanic population. Special attention to this population is warranted, especially since they are most likely to have larger tumors and/or metastatic disease at the time of diagnosis and to experience health disparities.[54]

**The Burden of Cancer in Puerto Rico**

Cancer is the second leading cause of death in the island, despite cancer-control efforts that have been present in Puerto Rico for decades. In 2004, 16.6% of all deaths were due to cancer.[2] Cancer, which is more predominant in men than in women,[2] touches almost every family. It was estimated that 54,000 patients were living with cancer from 1987 to 2006.[2] When addressing causes of cancer in Puerto Rican adults, Torres-Cintron, et al.[54] encouraged considering causes such as: tobacco use, western diet, physical inactivity, hormonal and reproductive factors, and occupational exposures. Improvements in prevention, detection, and treatment of cancers have led to a decline in the overall cancer death rate in Puerto Rico.[2] Despite these declines, patients with cancer continue to require significant resources.

Although monetary valuation of cancer does not take into account the psychological and emotional costs that cancer patients, their families, and society undergo due to the illness, accurate measuring of the economic impact of cancer is important for efficiently allocating limited resources with the aim to reduce the burden of cancer in society. According to the Pan American Health Organization**,** 20.4% of the Gross National Product in Puerto Rico corresponds to health expenditures.[2] This is twice as much as in Europe and 25% more than in the U.S. Therefore, cancer care is extremely costly and represents a great burden for Puerto Rico. For example, in 2006, the total cost of cancer in Puerto Rico was estimated to be $1.2 billion, including direct costs of about $396.8 million and indirect costs of about $805.5 million. In addition, cancer was the leading cause of years of potential life lost for both sexes combined, accounting for more than 12% of all premature mortality in Puerto Rico in the year 2005.[2]

Prostate Cancer is the most common type of cancer for men in Puerto Rico accounting for 40.6% of male cancer cases in 2010.[1] Alarming is the fact that according to the Cancer Control Plan,[2] each year approximately 2,050 men are diagnosed with invasive prostate cancer. It is also the leading cause of cancer deaths. The median age was 69 years at diagnosis for cancer of the prostate from 1999-2003, while the median age at death for men with cancer of the prostate was 82 years. Although the incidence rate of prostate cancer (127.9 per 100,000 men per year ) is lower in Puerto Rico than among U.S. Caucasians (169.2 per 100,000 men per year), after African Americans, mortality in Puerto Rico is much higher among Puerto Rican men with prostate cancer than in *all* other racial groups in the U.S. (U.S. Blacks 65.1%, Puerto Rico 36.4%, U.S. Caucasians 26.7%, U.S. Hispanics 22.1%, U.S. American Indian 18.3%, and U.S. Asian 11.8%; rates are per year and per 100,000 and age-adjusted to the 2000 U.S. standard population).[2] Taken together, these data suggest Puerto Ricans may experience an ethnic health disparity.

**Cancer Disparities**

Cancer disparities are adverse differences in cancer risks, incidence, prevalence, mortality, survivorship, and HRQOL among specific population groups.[2,55-57] Heath disparities may be attributed to multiple factors such as: acculturation, language barriers, changes in lifestyle, genetic factors, limited information available on genetic predisposition, lower education, health literacy, elevated exposures to environmental risk, limited access to health care, lack of health insurance, economical reasons, lack of knowledge about services, limited access to, and use of, cancer screening programs, and complex socio-cultural and geographic factors.[56-7]

The risk of developing cancer, and the risk of dying from cancer, increases with age. More than half (55%) of all cancers diagnosed in Puerto Rico occurs in people aged 65 years and older.[2] Individuals in this age group make up 13% of the population. This age group is estimated to increase to in 2025[7 2] and 68.6% of all cancer deaths in 2004 occurred in this age group.[2]

Disparities in health care persist for racial and ethnic minorities in the U.S. Numerous explanations for the observed ethnic disparity of Hispanic Puerto Ricans cancer incidence, treatment, and outcomes have been offered. Torres-Cintron et al.[55] suggests that disparities in the incidence and mortality of selected cancer in Puerto Rico might be explained by socio-economic position. Indeed, data on cancer incidence and mortality from the Puerto Rico Central Cancer Registry and data from the U.S. Census 2000 of the Puerto Rico socioeconomic position revealed that the incidence and mortality of cancer in Puerto Rico varied by socioeconomic position.[55] That is, municipalities with the lowest socioeconomic position (in the central region of Puerto Rico) had higher incidence and mortality from cancer of the esophagus and stomach. However, incidence for breast, colorectal, and prostate cancer were higher for areas of higher socioeconomic position (municipalities around the San Juan metropolitan area). An explanation provided by the authors of these differences in incidence was that this may be related to lack of access and use of medical care in the lowest socioeconomic position municipalities where fewer clinical facilities are available, which in turn may lead to under-diagnosis. Thus, the higher incidence of the most common cancers types in Puerto Rico in areas of higher socioeconomic position may be because there is better access to health care facilities (e.g., where most mammography facilities, urologist, and gastroenterologist are located).[55]

Other explanations include excess weight and physical inactivity among Puerto Ricans. Data from the Puerto Rico Heath Department show that in 2002, 58% of the population in Puerto Rico was overweight and 20% obese.[2] An estimated 20 to 30% of the most common cancers in Puerto Rico may be related to excess weight and physical inactivity.[2] Negron et al.[58] retrospectively reviewed the medical charts of 400 Puerto Ricans that underwent treatment for prostate cancer from 2003-2005, specifically to investigate if being overweight could affect both the sensitivity of the PSA as a diagnostic tool and the progression of prostate cancer among Puerto Ricans. Indeed, the percentage of overweight and obese men that had positive prostate biopsies was 35.38% and 38.13%, respectively, as compared to 26.15% of normal weight men. In addition, patients with BMI > 25 $kg/m^2$ had a 2.63 fold higher risk of metastases than those with normal BMI. Thus, obesity may play a role in explaining the higher mortality from prostate cancer in Puerto Rico. Similarly, Crespo et al.[59] studied the association between physical activity and prostate cancer mortality in Puerto Ricans with a randomly selected sample of 9,824 men age 35 to 79 years old who were followed for mortality for 40 years from 1965 until 2002. Physical activity did not predict prostate cancer mortality, the risk of prostate cancer mortality for the lowest level of physical activity was an OR of 0.99 (*95% CI* = 0.64-1.55).

One study examined how Hispanics ($N$ = 54) treated with EBRT for non-metastatic PC differ from their Caucasian ($N$ = 810) counterparts on biochemical disease-free survival.[60] These investigators found that Hispanic men treated with EBRT for non-metastatic PC tend to present with unfavorable disease characteristics (higher PSA levels and Gleason scores) and larger tumors, and showed a poorer 5-year biochemical disease-free survival rate compared to their Caucasian counterparts. Hispanic patients failed to reach a post-treatment PSA nadir of <1ng/mL seen in the other groups. This difference in survival was not explained by differences in follow-up. Moreover, more advanced disease requires more radical treatments that may lead to increased treatment-induced symptoms among of Hispanics. In contrast, Ho et al.[61] while examining cancer rates among island Puerto Ricans, mainland Puerto Ricans, and U.S. non-Hispanic whites from 1998-2002, found several statistical differences. They found that in men, from 9 of 14 cancer sites incidence rates were lowest among island Puerto Ricans, followed by mainland Puerto Ricans and non-Hispanic whites. The disparity between the two Puerto Rican populations was greater than that between mainland Puerto Ricans and non-Hispanic whites. Overall, cancer incidence rates for all sites combined were 34% lower in island Puerto Ricans compared to mainland Puerto Ricans and 16% lower in mainland Puerto Ricans compared to non-Hispanic whites (both $p$ values $< 0.05$).

In sum, culture may influence many aspects of health and healthcare. However, while examining health disparities, several authors addressed the importance of taking into consideration that Hispanics are diverse in nationality, exposed to different environmental factors, genetic composition, socio-economic status, culture, health outcomes, and, in the case of migration, patterns among subgroups are different.[61] With some exceptions, it appears that evidence exists that supports the notion that Hispanic oncology patients in general may be at a disadvantage as they have more socio-economic difficulties, and present with unfavorable disease characteristics and poorer 5-year biochemical disease-free survival rates when compared with non-Hispanic Caucasian counterparts in the U.S. What is not known is whether a second gap or source of disparities relates to issues of etiology, such as if there is a genetic predisposition and whether Hispanics also may perceive more treatment-related symptoms (pain, depression, and fatigue), self-image concerns, and worse HRQOL.[62] This study will contribute to knowledge about the interplay between genetics and symptom experience of prostate cancer patients in Puerto Rico.

**The symptom experience of cancer-related fatigue**

A symptom is a phenomenon subjectively experienced by individuals in situations of health and illness.[63] Although the patient's symptom experience has been the subject of study in chronic diseases, cancer is of special interest to nursing as research studies have demonstrated that symptom distress predicts survival, quality of life, and treatment intolerance in oncology patients.[63] Therefore, patients' decision to select treatment for non-metastatic prostate cancer is influenced by both understanding which treatment provides better survival and which treatment results in fewer negative symptoms.[49] The Hispanic culture sees male sexual dysfunction as a loss of sexuality and a public embarrassment.[62] Approximately one third of newly-diagnosed prostate cancer patients in Puerto Rico prefer EBRT.[49] We can speculate that this is due to the likelihood of impotence and erectile dysfunction accompanying prostatectomy. However, EBRT is not without side effects.[11]

Fatigue is one of many common side effects of radiation therapy (RT) that significantly affects HRQOL.[10,13] It often has been reported as one of the most distressing symptoms prostate cancer patients face during treatment.[64] The National Comprehensive Cancer Network defines CRF as a "distressing, persistent, subjective sense of tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and that interferes with usual functioning."[65] While the literature on CRF during treatment reports that approximately 80% of cancer patients will experience CRF during treatment, and 30% will continue to experience fatigue after a treatment regimen is completed, there is evidence of variability in the symptom experience of CRF.[64] For example, research has shown that some cancer patients report some degree of fatigue prior to the initiation of RT, and that the peak of fatigue during RT was at week five.[66] In contrast, two recent studies of patient assessments of CRF after RT showed significantly more fatigue only at the end of treatment that remained high at 6.5 weeks of follow up.[67-68] In addition, some patients persist with fatigue years after termination of RT.[11,36] However, others have reported that RT precipitated minimal to no fatigue in cancer survivors.[69]

There is no singular explanation as to why this variability in the symptom experience of CRF during RT exists. Some factors that have been proposed to contribute to or explain this variability include cancer diagnoses and patient's characteristics, such as age and gender, and or treatment characteristics. Hickok et al.[70] found that prostate cancer patients were least likely to report fatigue at the beginning of treatment when compared to breast, head and neck, lung, alimentary tract, or brain carcinomas. However, the Hickok et al.[70] study found that neither gender, age, nor total dose of RT predicted significant variance in fatigue severity. Other significant proposed explanations are: genetic vulnerability, hormonal and other biological factors; cognitive coping styles; and the occurrence of clusters of symptoms related to the disease itself or treatments that may interact and potentiate (synergistic) CRF.[6,13] Hence, researchers recommend a systematic and comprehensive evaluation of CRF during RT and as part of routine follow-ups.[13]

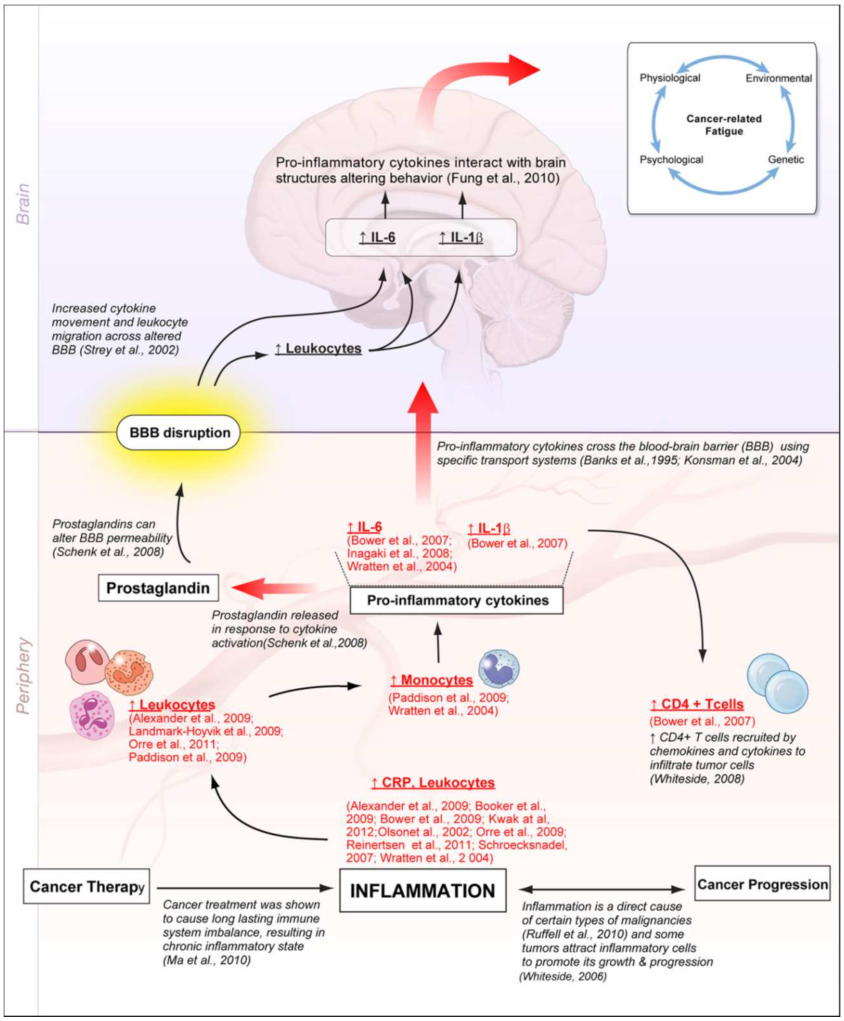
Research on the symptom of CRF has helped better understand the morbidity patients experience with RT for prostate cancer. Fatigue also could persist or recur after completed RT. Vordermark et al.[71]investigated the occurrence of chronic fatigue after RT using103 prostate cancer patients who were 2.1 years (median) after treatment. They found that 18.7% of patients reported that they were still suffering from severe fatigue, and that the symptom of fatigue significantly correlated with the occurrence of other symptoms such as fecal incontinence and urinary symptoms, but not with age. Lastly, not only is CRF a problem for patients, it also places a significant burden on the health care system.

Another important gap in the CRF literature is that no studies have examined the trajectory of fatigue, specifically among Hispanic or Puerto Rican men during EBRT, despite the availability of a valid and reliable Spanish version of the FACT-F.[72] Not only is there a need for more culturally competent research in this area, but because the Puerto Rican oncology population does not always report symptoms, risking under-assessment and under-management,[2,69] the need for this study is particularly necessary.

Lastly, despite the high prevalence of CRF and the associated negative outcomes, the etiology and mechanism underlying the symptom remain elusive. Both the Puerto Rico Comprehensive Cancer Control Plan (2008-2012) [2] and the 2013 Oncology Nursing Society Research Agenda [26] have called for more investigations on the incidence and etiology of CRF. While there is currently no optimal pharmacologic therapy and scant molecular evidence to guide the development of effective therapies for the management of CRF,[15] several pathophysiological mechanisms have been proposed to explain the development of CRF. Among these is the pro-inflammatory hypothesis.

**Pathophysiological mechanism of Cancer -Related Fatigue**

**Figure 3. The Association of Inflammatory Markers and Cancer-Related Fatigue.**




Source: Saligan, L. N., & Kim, H. S. [15] (Used with permission.)

The pathophysiological model underlying Figure 3 is based on the pro-inflammatory hypothesis that suggests that bio-behavioral symptoms such as CRF may be caused by dysregulated inflammation brought on by cancer or cancer treatments.[15] Briefly, it is well documented that the insult of cancer therapy (e.g., RT)[25] and certain types of malignancies (e.g., breast)[73] activate an initiation of molecular and cellular responses, including the expression of pro-inflammatory cytokines (e.g., IL-1β, and IL-6)[75-76] by white blood cells (especially monocytes).[15] Specifically, it has been found that RT in prostate cancer patients causes elevation in circulating levels of pro-inflammatory cytokines (IFN-γ, interleukin-6 (IL-6).[68] In addition, cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α) have been found to be associated with fatigue severity in breast cancer survivors, and interleukin-1 receptor antagonist (IL-1ra)[76-77] and c-reactive protein in testicular cancer survivors.[77] Based on this body of evidence, Saligan and Kim[15] proposed that "the systemic experience of CRF may be related to the interaction of pro-inflammatory cytokines and immune cells with brain structures that migrate through a disrupted blood-brain barrier altered by pro-inflammatory cytokines-related activities" (p. 16). However, EBRT affects many biological networks, including those related to immune response and mitochondrial function. Although the exact etiology of CRF has not been established, some evidence proposes that genetics plays an important role in the impairment of oxygen supply that is associated with EBRT-associated Fatigue.

**Biological Basis of EBRT-associated Fatigue**

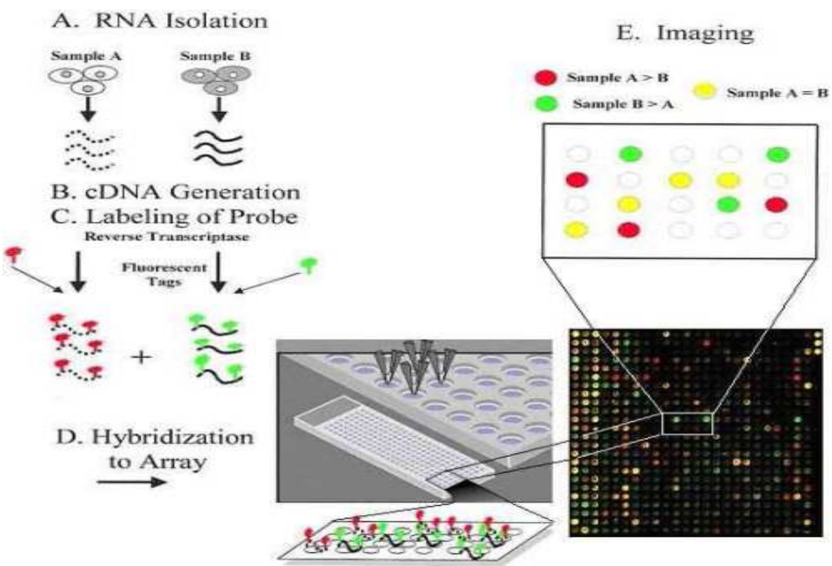
A biological basis for EBRT-associated fatigue proposes that ionizing radiation induces multiple cellular and biological effects by direct interaction with DNA or through the formation of hydroxyl radicals, leading to genetic instability. DNA damage induces a cellular response that includes activation of a number of signal transduction cascades.[78] There is increasing evidence that CRF is the result of ionizing radiation-induced gene expression in which respiratory function declines and the production of the ROS in the mitochondria increases, causing accumulation of mitochondrial DNA (mtDNA) mutation and oxidative damage and culminating in the metabolic shift from mitochondrial respiration to the glycolysis pathway for supply of adenosine triphosphate (ATP).[27] Since ATP is the energy source for cells, a reduction in ATP production can lead to an individual feeling fatigued.[16]

Further, mammalian genes are known to be activated in response to genotoxic stress such as ionizing radiation, including genes involved in cell cycle control or DNA repair, as well as transcription factors, growth factors and proteases.[27] As previously addressed, EBRT affects many biological networks, including those related to immune response and mitochondrial function.[16-19] Both of these mechanisms can contribute to the development of fatigue that compromises the HRQOL of cancer survivors. Therefore, a plausible etiology of EBRT-related fatigue is based on the fact that, in addition to reducing cell survival, ionizing radiation has been shown to simultaneously induce accelerated biological aging leading to cellular senescence containing dysfunctional mitochondria. In mammalian cells, mitochondria are the organelles that generate the majority of energy in the form of ATP via the respiratory chain and the oxidative phosphorylation system.[27-28] An estimated 90% of tissue oxygen and 1-5% of the mitochondria are metabolized to form the reactive ROS under normal physiological conditions and mitochondria are the immediate targets of the ROS generated in the organelles of tissue cells. Dysfunctional mitochondria are associated with the decline in mitochondrial respiratory function, excess production of the ROS and reactive nitrogen species, increase in the oxidative damage to mtDNA, lipids, and proteins in mitochondria, and altered expression of genes involved in intermediary metabolism. ROS may cause oxidative damage and mutations of mtDNA and alteration of the expression of several genes in tissues and senescent cells.[27,79-80] Thus, dysfunctional mitochondria induces detrimental cellular damage, not only to irradiated cells but also to cells of surrounding normal tissues that receive signals from irradiated cells, causing short and long term bystander effects through cytokine stimulation to respond to tissue damage. Genomic changes could mediate some of the effects of ionizing radiation. Therefore, to identify the molecular events associated with CRF, a number of investigators have used microarray technologies to examine how variations in gene expression patterns can play a part, or have a role, in the establishment of aberrant gene expression patterns among patients that culminate in experiencing severe fatigue.[15-17]

**Microarrays**

The Human Genome Project, first described in 1995,[81] provided the sequence of the entire human genome. Briefly, genes are contained in the DNA of the organism.[82] The mechanism by which proteins are produced from their corresponding genes is a two-step process: first, the transcription of a gene from DNA into a temporary molecule known as RNA; and second, translation, the step in which a protein is built using the RNA messenger as a blueprint. While there are other more recently developed high-throughput technologies, such as RNA sequencing, that use the capabilities of next-generation sequencing to reveal a snapshot of RNA presence and quantity from a genome at a given moment in time, the present study focused on microarray technology.[81] Gene expression microarray is a high-throughput technique that allows one to monitor the transcription step (DNA-to-RNA).[82] Molla, Waddell, Page, and Shavlik (n.d.)[82] summarized the process of conducting and interpreting gene-expression microarray. They explained that gene expression microarray provides extensive capabilities for the simultaneous assessment of the RNA expression levels of a large number of genes (thousands) or the whole genome. These authors further explained that the benefit of measuring RNA expression levels versus measuring the protein-production rate directly is that the latter is currently very difficult and impractical on a large scale. In contrast, researchers can measure the expression level of various genes by estimating the amount of RNA for that gene that is currently present in the cell. In addition, since the cell degrades RNA very quickly, this level will accurately reflect the rate at which the cell is producing the corresponding protein. Figure 4 illustrates a summary of the process of gene expression microarrays.

**Figure 4. Process Involved in Gene Expression Microarrays**




Source: http://www.fastol.com/~renkwitz/microarray_chips.htm

The process involved in gene expression microarrays, illustrated in Figure 4, shows that in order to find the expression level of a group of genes, one labels the RNA from a cell or a group of cells and spreads the RNA across a chip that contains probes for the genes of interest. For a human,[82] a single chip can only contain a subset of the genes present in the genome. To determine which genes are turned on and which are turned off in a given cell, when researchers runs a microarray experiment, an optical scanner records the fluorescence-intensity values (the level of fluorescence at each spot on the gene chip). Active genes generate a very bright fluorescent area; conversely, no fluorescence indicates that the gene is inactive.[81]

For the purpose of this study, the Affymetrix microarray technology was employed.[83] Affymetrix is a widely used RNA microarray that consists of pre-synthesized or in-situ synthesized oligonucleotide microarrays manufactured by Affymetrix (Cupertino, CA). The manufacturers developed a computer chip of oligonucleotides of approximately 25 base pairs in length that are synthesized in such a way that unique nucleotides representing a single human gene are present at every location on the array.[83] Microarray experiments can provide information on gene expression levels ranging from a few hundred to 50,000 independent genes for each sample, requiring biological, mathematical, and statistical skills to address and interpret the large volume of the obtained data. Even though several software algorithms have been developed to assist with the analysis of large datasets, they all rely on identifying genes that vary most over the samples being studied, with the purpose of discarding genes that do not change over experiments.[83] Microarrays have allowed an unbiased assessment of gene expression in the tissue of prostate cancer patients which has led to the identification of a large number of genes differentially expressed in response to treatments.

Some limitations of the use of microarray technology also have been identified, namely: heterogeneity (quantitative analysis of genes, while very informative, may not always be able to unravel the entire complexity of tissue damage processes in vivo); inability to detect isoforms and exon levels unless a specific kit is used to measure relative expression; and, cross platform comparison (there are inconsistencies between commercially-available platforms, making it difficult to compare independently obtained data sets addressing the same biological mechanism). Lastly, microarrays have been used to investigate the complex molecular response of cells and tissues to radiation, including identifying possible targets to ameliorate EBRT side effects such as fatigue.[79-80] The proposed study focused on a possible biological basis of EBRT-associated fatigue.

**Gene Expression and Cancer-Related-Fatigue**

*In vivo* studies have shown that differential expression of genes activating several physiological pathways may explain the mechanisms behind CRF.[15-19] Only a few studies have explored the use of genomic technologies to identify possible biomarkers of CRF,[15] and most of these studies have been conducted on White breast cancer survivors.[15, 84] In their review of literature on the association between immunogenomic markers and CRF, Saligan and Kim[15] identified seven cross-sectional studies exploring associations between levels of fatigue and genomic markers; all showed significant associations. However, important limitations in the interpretation of these findings were identified. These included use of small samples with further stratification of the samples during analysis, cancer patients and their family members for the analysis, and uncorrected *p*-values on the reported associations. Recently, Saligan et al. [16-19] found that changes in fatigue scores from Caucasian men with prostate cancer were significantly associated with changes in expression of mitochondrial-related and inflammation-related genes during EBRT.

Saligan and colleagues also described the relationships between mitochondria-related gene expression changes and self-reported fatigue in prostate cancer patients receiving EBRT[16]. These investigators used a prospective, exploratory, and repeated-measures design. Self-report answers to the Piper Fatigue Scale and peripheral whole-blood samples were collected with the PAX gene blood RA tubes from 15 patients at seven time points. Baseline data were compared against 15 healthy controls. The "Human Mitochondria RT2 Profiler PCR Array" was used to identify differential regulation of genes involved in mitochondrial biogenesis and function. With respect to fatigue scores, these investigator found that: (a) there was no significant difference in fatigue scores at baseline between patients and controls; (b) the mean fatigue score increased at midpoint of EBRT, slightly decreased at completion of EBRT, and remained slightly elevated at 30 days after EBRT; and (c) there was a significant change in fatigue score over time during EBRT compared with baseline data. Moreover, Saligan et al, selected human mitochondria genes to study based on the hypothesis that the inability of mitochondria to produce a sufficient supply of energy in the form of ATP plays a major role in fatigue. Their findings supported this hypothesis by demonstrating that 11 genes associated with mitochondrial integrity and functions critical to ATP production were differentially expressed during EBRT. Eight of the 11 differentially expressed genes were significantly associated with fatigue scores (*AIFM2, BCL-2, FIS1, IMMP2L, MSTO1, SLC25A23, SLC25A37, and SLC25A4*). Furthermore, three of the eleven genes (*BCL2L1, FIS1, SLC25A37*) were greater than 2.5-fold up-regulated. These investigators also found that genes ($n$ = 25) with more than a 1.5-fold change in expression at $p< .05$ at days 14, 21, 42, or 72,were associated with cellular pathways that were related to morphology, assembly, or cell organization, and cell death. Thus, this study provides beginning empirical evidence that genes related to mitochondria and their function are differently expressed during EBRT and are potentially associated with changes in fatigue symptoms among non-metastatic prostate cancer patients. The authors recommend a prospective study to validate and confirm the relationship of the expressed mitochondria-related genes with other co-variates to be included, such as race, disease severity, depression, and symptom clusters; and that studies with a larger sample size are needed.[16]

Saligan and colleagues[17] also investigated the association of CRF with the activation of inflammatory and neuroprotective pathways among 16 patients (Caucasians $n$=10, African American $n$=2, and others $n$=4) receiving EBRT for non-metastatic prostate cancer. Fatigue scores from the Piper Fatigue Scale and blood samples were obtained at baseline (prior to EBRT, D0), at one hour following initiation of EBRT (D1), on day 7 (D7), on day 14 (D14), at midpoint (days 19-21, D21), at completion (days 38-42, D42), and four weeks post-EBRT (days 68-72, D72). Gene expression profiling was determined using microarray analysis from peripheral blood. Compared to baseline mean scores, the mean fatigue scores significantly increased at midpoint of EBRT, continued to be significantly higher than baseline at end of treatment, but showed no significant difference from baseline to one month post-EBRT. Notably, the most differentially-expressed genes were related to inflammation, namely: interferon alpha-inducible protein 27 [*IFI27*], B-lymphocyte antigen CD20 *[MS4A1*], Ig mu chain C region [*IGHM*], C-C chemokine receptor type 7 [*CCR7*]), and iron synthesis (carbonic anhydrase 1 [CA1], hemoglobin subunit delta [*HBD*], hemoglobin subunit gamma-2 [*HBG2*], alpha hemoglobin stabilizing protein [*AHSP*], iron-sulfur cluster assembly 1 homolog [*ISCA1*]), and Alpha synuclein[ *SNCA]*. The investigators selected *SNCA* for further investigation in this study because of its known association with neuro-inflammation. The *SNCA* gene had a 2.95-fold change in expression at D21 compared to baseline. Also, fatigue scores were significantly correlated with *SNCA* gene expression on D14 and plasma α-synuclein concentrations on D42 of EBRT. The canonical pathways related to *SNCA* over-expression during EBRT using Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com, Redwood City, CA) revealed pathways related to 14-3-3-mediated signaling, which is involved in phosphorylation-dependent protein-protein interactions. One possible explanation provided by the researchers on over-expression of the *SNCA* in this study was that it may be a part of a physiologic response to intrinsic or external stressors (i.e., EBRT), a-synuclein is expressed to serve as a neuro-protective mechanism against subsequent insults. If so, neuro-inflammatory mechanisms may play a role in the development of fatigue. Another important contribution of this study was the need to explore the role of other networks involved in fatigue development, such as inflammation and iron-synthesis, because genes related to these networks were observed to be differentially expressed in this study.[17]

Saligan's group[19] further studied the association between interferon alpha-inducible protein 27 (*IFI27*) expressions and fatigue intensification during EBRT for non-metastatic PC. *IFI27* was the most up-regulated gene (expression value = 0.774, $p<0.0001$) among fatigued men with non-metastatic prostate cancer receiving EBRT in their earlier study.[17] Peripheral blood samples and fatigue scores were collected at three time points (prior to EBRT, at midpoint, and at completion of EBRT) from 40 Caucasian men (20 on EBRT and 20 matched control participants on active surveillance). Participants answered the Piper Fatigue Scale and the PROMIS fatigue scale. Compared to baseline, the mean fatigue scores increased significantly at midpoint and at completion of EBRT; however, there was no significance difference from midpoint to completion of EBRT. Significant up-regulation of *IFI27* was confirmed, both via qPCR and (ELISA) techniques. Also, *IFI27* gene expression increased significantly from baseline to midpoint and from baseline to completion of EBRT. Lastly, a significant association between changes in fatigue scores and *IFI27* gene expression using qRT-PCR was observed from baseline to midpoint of EBRT, but no significant association was obtained between changes in fatigue scores and changes in the expression of *IFI27* gene from baseline to completion of EBRT. One possible explanation for up-regulation of *IFI27* gene provided by the authors was that *IFI27* is a gene known to induce apoptosis, and is highly induced by interferon-alpha (*IFN-α*) and interferon beta (*IFN-β*), both cytokines that alter immune-response through activities of T lymphocytes and dendritic cells.

Thus, in order to capture the initial inflammatory response of EBRT, which peaks at midpoint (day 21),[15-19] in this study only levels of the differentially expressed genes at baseline and at midpoint of EBRT will be determined. Saligan and colleagues' findings raise important information that fatigue intensification during EBRT may be a bystander response to radiation, and that this bystander response could be explained by up-regulation of *IFI27*, which influences mitochondrial function and immune response, both of which are mechanisms proposed to be related to CRF. A genome-wide profiling using Microarray technology also has been useful in identifying genes associated with Interferon-alpha (*IFN-alpha*)-induced depression and fatigue treatment for chronic hepatitis C (e.g., *OAS2* (*ILMN_2248970*)[85] and chemo-radiation for head and neck cancer- induced oral mucositis (e.g., *Dkk-1 gene*).[86]

Unlike prior investigations that used microarray technology, Saligan and colleagues[18] used proteomics technology (measures protein directly) to investigate changes in expression of novel proteins with changes in fatigue symptoms of 12 non-metastatic prostate cancer patients receiving EBRT. Proteomic-based techniques have been used to identify several biomarkers to diagnose many types of cancer and identify proteins that are involved in the underlying mechanisms of symptoms. Fatigue scores were measured using the FACT-F sub-scale. Measures were collected at baseline (before EBRT) and at midpoint (Day 21) of EBRT. Depleted sera from both time points were analyzed using two-dimensional difference gel electrophoresis, and up-/down-regulated proteins were identified using liquid chromatography-tandem mass spectrometry. Western blot analyses confirmed the protein changes observed. In this study participants were grouped according to the change in fatigue scores during EBRT: the high fatigue (HF) group were those with increasing fatigue symptoms (declining FACT-F scores) from baseline to midpoint of EBRT, and the no fatigue (NF) group were those with no change or with increasing FACT-F scores between the two time points. Results showed that three subjects were categorized in the NF group (mean FACT-F score: baseline = 44.0 $\pm$ 8.0, midpoint= 46.3 $\pm$ 9.0) and nine subjects were categorized in the HF group (mean FACT-F score: baseline = 47.0 $\pm$ 2.9, midpoint = 36.8 $\pm$ 5.5). No differences in hematocrit levels and depression scores were noted between the fatigue groups at baseline and at midpoint of EBRT. The most notable finding was that *ApoA1* levels were observed to be higher in HF subjects than in NF subjects at baseline, more significantly at midpoint of EBRT than at end of treatment. Two important explanations were provided.[18] First, apolipoproteins play a role in cholesterol transport and have been associated with several important physiological processes necessary to maintain immune regulation and cognition. Second, the apolipoproteins also have been found to be involved in proteolytic breakdown of beta-amyloid in Alzheimer's disease. The authors explained that increasing *ApoA1* protein levels during EBRT in this clinical population is a protective mechanism to counter an acute stressor to prevent neuro-inflammation and decrease proinflammatory cytokine production, in which both mechanisms were suggested to potentially explain the etiology behind RF. An additional finding was that *TTR* or pre-albumin (a negative acute phase protein; its concentration is reduced during acute phase response) was the third differentially expressed protein, whose level was observed to be lower in HF subjects than in NF subjects. In other words, the differential expression of these three proteins (*ApoE, ApoA1, TTR*) is linked with two mechanisms that are known to be associated with CRF: neuro-inflammation and proinflammatory cytokine production.[18]

Lastly, genome-wide profiling using microarray technology also has been useful for identifying genes associated with Interferon-alpha (*IFN-alpha*)-induced depression and fatigue treatment for chronic hepatitis C (e.g,.*OAS2 (ILMN_2248970*)[85]and chemo-radiation for head and neck cancer-induced oral mucositis (e.g,.*Dkk-1 gene*).[86] Genome researchers also have evaluated the associations of single nucleotide polymorphism (single positions of variation in DNA) of inflammation-related genes including (*IL1B, IL6, and TNFα*) with changes in fatigue in PC patients receiving other type of treatment such as ADT [43] and apoptosis-related genes (*BDNF Val66Met*) in breast cancer survivors (6 months or more post-treatment).[87] The former demonstrated that prostate cancer patients treated with ADT who carry alleles of the *IL6* and *TNFA* genes are susceptible to developing severe fatigue.[43] The latter study showed that, among breast cancer survivors (6 months or more post-treatment), the presence of the BDNF Val66Met SNP biomarker was related to lower symptom scores, but the effect size was small and the relationship did not persist when controlling for confounders.[87] Heinz[87]cites Kim, Barsevick, & Tulman[88] who found that low levels of *BDNF* have been associated with ROS that result in oxidative stress leading to cell death [apoptosis] processes that have been linked to a variety of cancer-related symptoms.

In sum, it appears that genetic variation plays a role in the development of CRF during treatment. Findings from the above studies also provide support for the value of microarray in understanding regime-related toxicities other than CRF, and the future of the use of genetic testing as a tool to predict toxicity risk. In addition, researchers have found that CRF is a symptom with multiple factors contributing to its presentation and outcomes.[13,15,64] Relevant studies that identify physiological, psychological, and situational factors that interact and influence the symptom experience of CRF are presented next.

## Other Factors Influencing Cancer-Related Fatigue

### Situational factors

Situational factors are aspects of the social and physical environment that need to be considered when examining CRF since they may affect the individual's experience and reporting of symptoms.[30] Servaes, Verhagen, and Bleijenberg's[36] review of literature on prevalence, correlates, and interventions for CRF during and after cancer treatments revealed that in 9 out of 10 studies marital and working status were not found to be significantly related with CRF. The exception study showed that in mixed-diagnoses cancer patients, increased general fatigue during RT was found to be significantly associated with being in a de-facto relationship (not legally married) when compared with being legally married or single ($p < .05$, 2.21, (0.74-3.67)). Fatigue also was significantly associated with unemployment ($p < .05$, 2.04 (0.44, 3.63)), but not significantly associated with education. Other researchers have documented that non-metastatic prostate cancer patients experienced worsening of CRF from 4 to 10 years after EBRT as well as financial difficulties.[89] The participants' situational factors of employment status, marital status, and education were used to describe the sample in the present study.

**Psychological factors**

CRF is a multidimensional symptom that also is influenced by psychological factors. Several studies have documented that depression and anxiety were significantly correlated with fatigue. Purcell et al.'s[90] study of 210 patients receiving RT for mixed-cancer diagnoses found that increased general fatigue and increased physical fatigue were found to be significantly associated with depression ($p = .05$, 0.41, $CI$ = 0.00-0.27; $p < .01$, 0.27, $CI$ = 0.14-0.36, respectively.) However, the relationship between anxiety and general fatigue and physical fatigue was not statistically significant. Irvine et al.[91] study on prevalence and correlates of fatigue among 101 patients receiving treatment with either radiotherapy or chemotherapy found that mood disturbance was one of the strongest correlates of fatigue ($r = 0.47$, $p < .0001$). Redeker et al.[92] study among 263 cancer patients receiving chemotherapy found that fatigue, depression, and anxiety were positively correlated with one another ($r = 0.26$ to $r = 0.69$, $p < 0.001$). Similar findings in a mixed group of cancer patients during cancer treatment were reported by others.[33,91] These findings provided support to measure depression in the current study. Overall, these results are consistent with the contention that CRF among patients during RT is related to the psychological factors of anxiety and depression.[91]

**Physiological factors**

Physiological factors include normally functioning body systems and the occurrence of any pathology, including cancer.[30] A variety of physiological factors have been postulated to be associated with RT-associated fatigue. For example, studies have described a negative correlation between general fatigue during RT and age ($p = .05$, -0.07 [-0.11, -0.03]),[90] but other researchers have found that neither gender nor age was predictive of fatigue severity at any time point.[36,66] In a prospective study of 55 non-metastatic prostate cancer patients treated with RT, researchers found that treatment-related symptoms, including fatigue, were more likely to worsen if baseline PSA values, Gleason score, and CTV2 (tumor stage) were above the desirable limits.[36] However, as in other studies,[36,66] Troung et al.'s [66] study of 28 prostate cancer patients having EBRT for non-metastatic prostate cancer found that fatigue was not significantly associated with the participant's T-stage, Gleason score, baseline PSA, number of RT fractions and number of RT fields, or total RT dose. Hickok et al.[70] also identified the need to assess the presence of co-morbid physical illnesses, such as hypothyroidism or congestive heart failure, as contributing factors to CRF.

While anemia is believed to be related to CRF, during RT it may be less significant as the erythropoietin system effectively compensates by proliferating upon demand.[93] A study of 2,111 complete blood counts (CBC) from 299 cancer patients showed no statistically significant decrease in total red blood cells (RBC) during and at the end of RT, or any clinically significant anemia.[93] However, Wratten et al.[74] found that higher baseline fatigue level and higher baseline neutrophil and RBC counts were the most predictive factors for fatigue during radiotherapy for breast cancer.

**Additional covariates of fatigue**

Meta-analysis and a Cochrane report have provided evidence that randomized controlled trials on exercise, which improves aerobic capacity, muscle strength, body composition, and physical functioning, have been shown to reduce fatigue.[94-95] Windsor et al.[96] tested whether an aerobic exercise intervention during EBRT for prostate cancer would reduce the incidence of fatigue. The investigators found that moderate-intensity walking produced a significant improvement in physical functioning with no significant increase in fatigue and that men in the control group had significant increases in fatigue from baseline to the end of radiotherapy. Similar findings in a mixed group of cancer patients during RT were reported by Mustian et al.[97] After a 4-week home-based aerobic and resistance exercise training, participants in the intervention group reported significant lower fatigue at the end of radiotherapy and at three months. These findings suggest that it is possible that increasing physical activity during EBRT may decrease worsening of fatigue.

Ample evidence exists on the relationship between sleep disturbance and CRF during cancer treatments. Sleep disturbance has been found to be positively correlated with fatigue,[12,36,90] to exacerbate CRF,[98] and, to be a significant predictor of fatigue.[12,99] For example, Miaskowski et al.[100] found that higher sleep disturbance was one of the predictors of baseline levels of morning fatigue in prostate cancer patients who underwent EBRT. Dhruva et al.[101] reported similar findings among breast cancer women who underwent RT.

**Disparities in Symptoms Experience**

While CRF during EBRT appears unrelated to age, cancer stage, radiation dose/fraction, it is frequently associated with ethnic differences. Symptom management inequities also are evident.[20, 4-5] Specifically, Hispanics are more likely to experience delays in diagnosis and to receive less than optimal treatment plans than Caucasians.[14] Multiethnic cohort research studies suggest that Hispanics are more likely than their Caucasian counterparts to experience treatment-related symptoms, including fatigue.[7] For example, in their study of 116 breast cancer survivors, Eversley et al.[14] reported that Hispanic women showed significantly higher rates of fatigue and depression than Caucasians. Similarly, in a research study of 139 breast cancer survivors, fatigue was the most common (76%) symptom.[20] When comparing ethnic groups, Hispanics were significantly more likely than Caucasians to report more than 10 symptoms even after adjusting for several covariates. Being Hispanic, or older than 65 years old, or unemployed, was related to reports of chemotherapy-related symptoms. Pain-related symptoms also were significantly reported by Hispanics and those older than 65 years. A longitudinal study[7]on early referral to a supportive care specialist for symptom burden in a study of 752 lung cancer patients showed that Hispanics reported higher rates of fatigue, pain, depression, and swelling and that fatigue was significantly associated with referral for symptom management. In addition, no significant improvement in pain or fatigue was observed among Hispanics in general when compared to Caucasians.[7]

Regardless of the documentation of disparity in the symptom experience of CRF reported by Hispanic groups, few CRF studies have included prostate cancer patients during EBRT, particularly Puerto Rican participants, as a separate group in their analysis. This investigator conducted a descriptive study with 50 Puerto Rican cancer participants reporting their symptoms and self-care methods used to alleviate their symptoms during treatments from August to October, 2010.[25] Participants were 64% female, had a mean age of 56.6 years; 37 had chemotherapy alone and 13 had RT. The diagnoses mostly were breast, cervical, colorectal, and prostate cancer. All the symptoms in the fatigue subscale were reported: feeling sluggish (56%), difficulty sleeping (54%) and depression (46%). The Therapy Related Symptom Checklist (TRSC) scores correlated with Symptom Alleviation: Self-Care Methods ($r = 0.82$; $p < .001$) and with HRQOL ($r = -0.37$; $p < .01$), indicating that the higher the total TRSC scores (indicating more frequent and more severe symptoms), the lower the reported HRQOL scores, and the lower the functional status rating. The Puerto Rican prostate cancer participants' ($N$=13) characteristics included: mean age of 55 (range 42-69); Karnofsky Performance score mean of 87.6 (range 60-100); HRQOL mean 8.3 (range 7-10); 23% with less than a high school education; 38% who were currently working; and 80% who reported feeling sluggish.

The literature on pain is consistent with the CRF literature; the occurrence and severity of pain among non-Hispanic African-Americans (*OR*= 1.78; *99% CI*, 1.33-2.37) and Hispanics (*OR*= 1.80; *99% CI*, 1.26-2.56) is higher compared with that among Caucasians.[69,102-3] This suggests there may be a greater need for symptom surveillance, treatment and control for these subgroups. In another study, one notable finding was that health care providers under-recognized and under-treated cancer-related-symptoms, especially among Hispanic women.[104] Thus, Hispanic cancer patients in general have a higher risk of adverse effects from treatment and a need for more targeted interventions.[14] These findings have important ramifications for current theory regarding the etiology of disparity in the symptom experience of CRF.

Numerous explanations for the observed ethnic disparity have been offered. These include level of acculturation, quality of information regarding therapeutic interventions, ability to afford rehabilitative therapies, language barriers, effectiveness of communication with providers, culture, and socio-economic factors.[14, 20] Much remains to be investigated, including whether physiological evidence can explain the variability of fatigue responses to EBRT between ethnic groups. Indeed, others have proposed focusing on biological causal pathways to explain the variability in the symptom experience of fatigue.[21-24]

**Biological vulnerability to disparity in symptoms experience**

While the etiology and mechanism underlying disparity in the experience of CRF remains elusive, findings from recent studies on the symptoms of pain and depression that propose intrinsic biological vulnerabilities as plausible explanations, may apply to CRF.[24] Contemporary researchers[24] have proposed that inflammation, particularly from *IL-6, IL-8* and *TNF-α*, may be a common biological mechanism causing variability in self-report of cancer-related pain and that *IL-6, IL-8* and *TNF-α* may be potential targets for treating pain. For example, Reyes-Gibby et al.[24]examined the extent to which functional polymorphisms in *TNF-a -308 G/A,IL-6 -174G/C*, and *IL-8 -251T/A* are associated with the severity of pain among a sample of 446 Caucasians, 125 African-Americans, and 35 Hispanics with newly diagnosed lung cancer (untreated patients). These researchers observed that African-Americans (31.5%) had the highest proportion reporting severe pain, followed by Hispanics (20%) and Caucasians (17%; $p< 0.001$), but no significant association was obtained between genotypes in *TNF-a, IL-6,* and *IL-8* and severe pain for either African-Americans or Hispanics. In a review of literature on gender disparities in depression, Accortt, Freeman and Allen[105] found evidence that supports the premise that biological factors may contribute to explaining this disparity. A study with adolescents[106] also found gender differences in the effects of *5HT (2A)* (human serotonin-2A receptor gene), *TPH* (tryptophan hydroxylase), and *5HTTLPR* (serotonin transporter gene promoter polymorphism) in genetic vulnerability to depression. In contrast, a similar study with adults found no differences in the genotype frequency between genders.[105,107] Importantly, researchers reported that the empirical evidence for gender-specific vulnerabilities to depression does not suggest that this disparity in depression rates is due to differential symptom reporting.[8] Further research is needed to clarify the contributions of biological factors to disparities in the symptom experience of fatigue because that may lead to genetic discoveries with more target therapeutic implications.

Saligan et al. found changes in fatigue scores among Caucasian men with prostate cancer were significantly associated with changes in expression of mitochondrial-related and inflammation-related genes during EBRT.[16-19] No similar studies have looked at the associations of changes in gene expression and fatigue symptoms in Hispanic PR men during EBRT. Showing no difference in physiologic profiles associated with fatigue intensification during EBRT among ethnic groups will bridge this gap.

## Summary

In summary, Gonzalez preliminary study in 2011 showed that Puerto Rican men with prostate cancer (*N*=13) have a higher prevalence (80%) of fatigue at midpoint of EBRT.[25] Despite variation in symptom reporting among ethnic groups, it is well documented that

Hispanic P.R.'s are an understudied population who are underrepresented in clinical trials, especially in symptom research.[2] While the etiology and mechanism underlying this disparity in symptoms experience remains elusive, recent studies propose intrinsic biological vulnerabilities as a plausible explanation.[21,14] The current study bridges that gap by comparing gene expression patterns of Hispanic Puerto Rican men treated with EBRT with Saligan's findings among Caucasian men receiving the same treatment. Gene expression measured from RNA is tissue specific.[83] Microarray technology can provide an unbiased approach to investigating potential biological pathways associated with specific conditions, including symptoms such as fatigue. Saligan and colleagues recently found significant associations between differential expression of eight mitochondria-related genes and changes in fatigue among Caucasians receiving EBRT for non-metastatic prostate cancer.[16] They also found a significant correlation between changes in fatigue and the up-regulation of α synuclein during EBRT, providing preliminary evidence of the role of neuro-inflammation in the development of CRF.[17] This investigator employed a similar microarray technique to explore the differential expression of genes from peripheral whole blood RNA collected from Hispanic Puerto Rican men at baseline and at midpoint of EBRT. Functional networks of the differentially expressed genes were examined using Ingenuity Pathway Analysis to determine pathways that may explain the possible physiological mechanisms that influence fatigue intensification during EBRT in this population.

# CHAPTER III

# METHODOLOGY

## Research Design

The present study is a prospective exploratory and comparative study designed to describe and better understand the fatigue experienced by Hispanic Puerto Rican men over the course of EBRT. Microarray technology was employed to explore the differential expression of genes from peripheral whole blood RNA collected at baseline and at midpoint of EBRT. Functional networks of the differentially expressed genes were examined to identify pathways that may explain the physiologic underpinnings of fatigue and fatigue changes during EBRT treatment for prostate cancer. To accomplish the proposed objectives of the research study, three aims were proposed: (a) assessment of fatigue and changes of fatigue overtime (Aim 1); (b) assessment of gene expression overtime (Aim 2); and (c) relationship between gene expression changes and fatigue changes (Aim 3).

### Sample

The sample for the proposed study included 26 Hispanic Puerto Rican men with non-metastatic prostate cancer who received EBRT. Study participants were recruited from the ambulatory Radio Oncology Center at the "Clinica Las Americas." After obtaining Human Subjects Committee approval from both the University of P.R. Human Subjects Committees and the KUMC Institutional Review Board (IRB), the principal investigator (PI) recruited participants for the proposed study from patients being evaluated to receive EBRT for non-metastatic prostate cancer.

**Participants' Inclusion Criteria**

The **inclusion criteria were**: Hispanic PR males over 40 years of age with clinical diagnosis of non-metastatic prostate cancer, who were scheduled for EBRT. Eligible participants also needed to be able to read and write at the 6th grade level and to provide written informed consent.

**Participants' Exclusion Criteria**

**Exclusion criteria** included: progressive or unstable disease of any body system causing clinically significant fatigue; systemic infections (e.g., human immunodeficiency virus, active hepatitis); documented history of major depression, bi-polar disorder, psychosis, or alcohol dependence/abuse within the past five years; uncorrected hypothyroidism or anemia; second malignancies; concurrent chemotherapy with EBRT; and those with chronic inflammatory disease that may alter pro-inflammatory cytokines profiles (e.g., rheumatoid arthritis). Additionally, patients taking sedatives, steroids, or non-steroidal anti-inflammatory agents were excluded because these medications are known to affect immunogenetic changes.[16] Inclusion and exclusion criteria were verified by patient interview.

**Sampling procedure**

A radiation oncologist at the Clinica Las Americas Tomé & Ubiñas Radio Oncology Center facility assisted with recruitment of participants for the study. To address concerns about educating staff about the protocol, the investigator scheduled a short presentation for faculty and staff of the RT unit to explain the study and the flyers (Appendix B) with study details placed at the RT facility. The radiation oncologist informed potential participants about the study and introduced interested patients to the investigator, who was available at the facility. The investigator’s responsibilities with the participants' included: (a) providing information about the study, (b) verifying inclusion criteria, (c) obtaining informed consent, and (d) coordinating study data collection dates and times (at participants’ convenience).

**Setting**

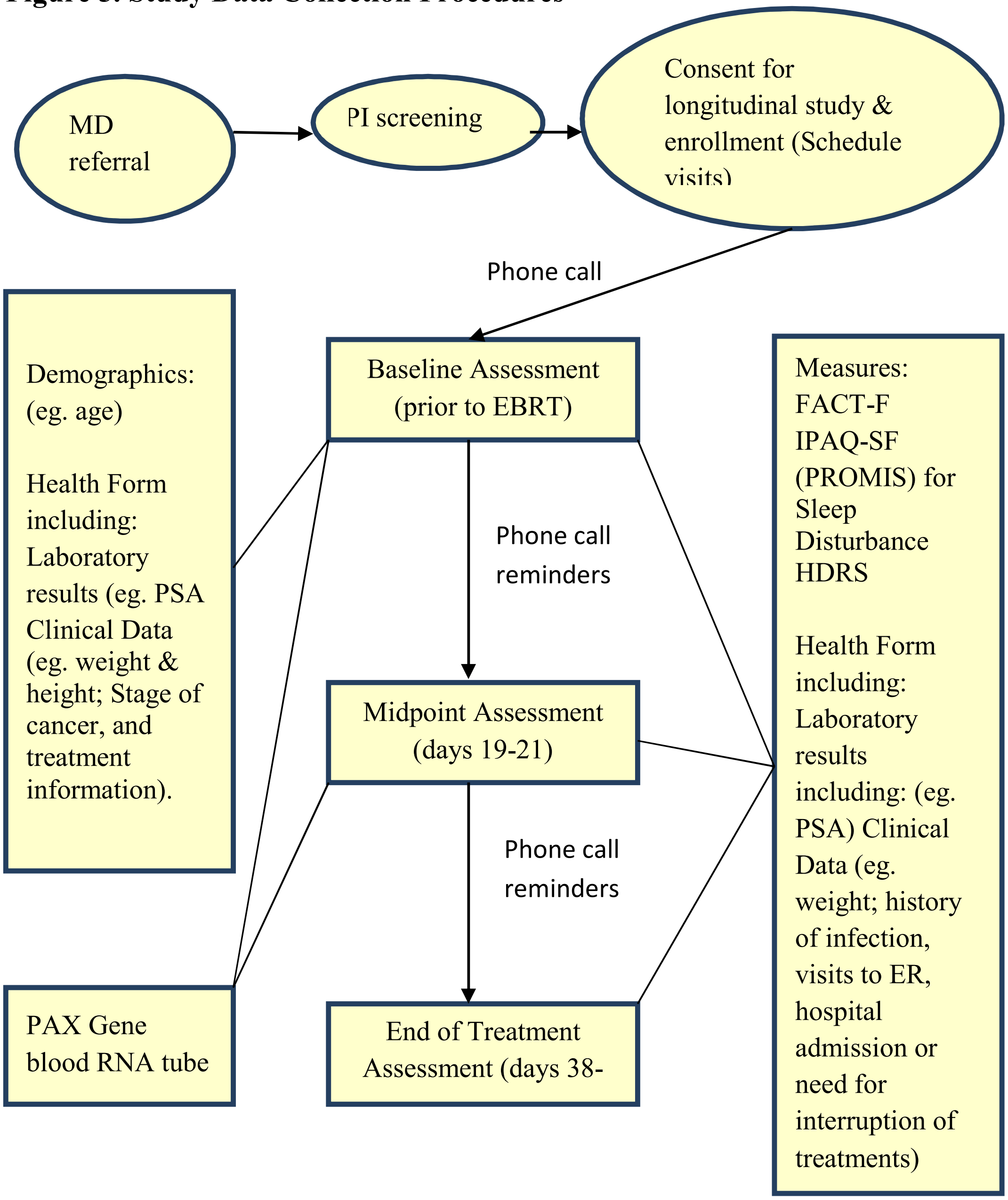
Recruitment and data collection of study participants took place at the ambulatory Radio Oncology Center at the “Clinica Las Americas.” The Tomé & Ubiñas Radio Oncology Center is a free standing facility located in San Juan, Puerto Rico, approximately 3 miles from the University of P.R. Medical Sciences Campus. The facility is housed in two separate buildings providing office space and treatment space inside the main medical office compound called "Clinica Las Americas." The radiation oncologist provided a designated private interview/examination room and, after obtaining patient consent, access to participants’ medical chart, to verify the inclusion and exclusion criteria and to obtain selected heath information.

## Procedures

The procedures section begins with a figure that shows a summary of the study data collection procedures. Next, a detailed discussion of participant recruitment and what happened at baseline, midpoint, and end of treatment visits is presented. This is followed by a discussion of the study variables and measurements, statistical analyses, and pitfalls and resolution for each aim. At the end of the chapter, the ethical considerations are discussed.

The data collection process included four visits: recruitment, baseline, midpoint and end of treatment (see Figure 5).

**Figure 5. Study Data Collection Procedures**




**Subject Recruitment**

Prior to beginning data collection, approval by the Human Subjects Committee of both the Midwestern academic medical center and University of Puerto Rico was granted (see Appendix C). Prior to EBRT, patients were scheduled for two visits at the RT facility, one for evaluation and one for simulation. Evaluation days occurred approximately 14 days prior to simulation (Monday and Wednesdays afternoons), and during simulation, which is 14 days prior to initiation of treatment (Tuesdays and Thursday mornings).

Subject recruitment occurred on evaluation days after the physician evaluation. The enrollment process occurred using the following steps: (a) patients who were likely to meet the inclusion and exclusion criteria were approached by the radiation oncologist who had knowledge of those criteria; (b) patients were informed about the research study investigating fatigue in prostate cancer patients under EBRT; (c) patients were asked them if they were interested in getting more information; then, (d) the staff introduced those patients interested in receiving more information to the PI who was available at the facility and interviewed them in a private room at the RT unit. Patients interested in participating in this study were screened by the PI for eligibility with a brief health history interview that included summary questions about cancer disease characteristics, previous and planned cancer treatments, concomitant medications, co-morbid conditions, systemic infections, and documented history of major depression, bi-polar disorder, psychosis, or alcohol dependence/abuse within the past 5 years (see Appendix D). The radiation oncologist referred a total of 31 participants interested in receiving more information. Of these 31 patients, three were not interested in participating. It is unknown how many were not interested in receiving any further information.

Eligible participants were formally asked if they wanted to participate in the study, which involved self-report questionnaires and blood draws, and if they permitted, selected chart review. They were given a written informed consent form (see Appendices E & F) to sign after they indicated their understanding of the study procedures and willingness to participate. All patients interviewed by the PI were assured that their decision to participate would not affect their care in any way. The recruitment took approximately three months. After signed informed consent, the PI scheduled the three study data collection dates and times. All study procedures at baseline (prior to EBRT), midpoint (days 19-21) and completion (days 38-42) of EBRT were scheduled to take place at the participants' convenience. Thirty-one individuals were approached for possible participation in the study; three refused participation, 28 agreed, and 26 of these were eligible and gave their informed consent. The reasons for those that refused to participate were: "agreed to answer questionnaires but not to give blood" (2 patients) and "not interested" (1 patient). Two were ineligible due to diagnosis of chronic renal failure.

**Baseline Visit**

On baseline evaluation days, the demographic information on the demographic form (see Appendices G & H) was obtained from study participants. The PI reviewed with the study participants the instructions on how to fill out each questionnaire. The following self-report instruments (discussed below under Measures) were completed by study participants: the validated Spanish-versions of the Fatigue sub-scale from the Functional Assessment of Cancer Therapy-Fatigue (FACT-F) (see Appendices I & J), the International Physical Activity Questionnaire Short Form (IPAQ, SF) (see Appendices K & L), and the PROMIS-sleep disturbance questionnaires (see Appendices M & N). The PI then administered the Hamilton depression scale (HDRS) (see Appendix O), validated in Spanish speaking populations, to obtain depression scores.

No participants experienced psychological distress from answering the fatigue, IPAQ, PROMIS-sleep disturbance questionnaires or from the HDRS, or were observed to have increasing depressive symptoms. A clinical psychologist, and director of the Behavioral

Sciences Research Institute, University of P.R., advised and trained the PI in the process of: (a) using and scoring the HDRS; (b) recognizing signs and symptoms of depression and sleep disturbance; and (c) referral to further resources. Since no participants scored 15 or more on the HDRS at any time point during the study, there was no need to refer to the clinical psychologist for further evaluation for depression. An increase in HDRS score during the study was not a criterion for withdrawal of subjects from participation. Combined participants' self-report of demographics and administration of questionnaires and the PI's rating on the HDRS required less than 30 minutes of the participants' time.

After completing these forms, participants were measured for height and weight (required for BMI calculation) using standardized techniques in a private location. This was followed by the PI taking a whole blood sample for RNA analysis. Blood samples were managed and transported in accordance with OSHA regulations and University of Puerto Rico policies. The blood drawing, height and weight measuring required approximately 15 minutes of the participants' time. Participants were informed about the molecular analysis that was conducted on their blood and they were given the option of learning about laboratory results of the blood tests as approved by both Human Subjects Committees. As per University of Puerto Rico policy, if medical care was necessary as a result of the blood drawing, it would have been provided free of cost by the University of Puerto Rico Medical Center. This was not necessary for this study's participants.

Following baseline study procedures, the PI reviewed each subject's medical chart for recording selected clinical information on the health form (see Appendix P) and entering into the data base. Unique identifiers were assigned to each participant. The unique identifier was used to match demographics and questionnaire data with the blood samples and medical chart data.

**Midpoint Visit**

Midpoint visit procedures were similar to baseline. Participants once again completed the FACT-F, IPAQ, and PROMIS-sleep questionnaires. In addition, participants' BMI and blood sample were obtained. Lastly, the PI rated the HDRS and gathered other clinical characteristics of participants (e.g., infections, CBC) by chart review.

**End of Treatment Visit**

The procedures at the end-of-treatment visit were the same as at midpoint with one exception. No blood samples were drawn at this visit.

## MEASURES

Variables, method, and frequency of measurement for each variable are summarized in Table 1. A detailed description of each variable is provided below.

Table 1
*Summary of Measurements*

| Variables | Method of Measurement | Frequency |
|---|---|---|
| Cancer-Related Fatigue | FACT-F | Baseline, mid-point, and end of treatment |
| Gene Expression levels of peripheral blood cells | PAXGene blood RNA tube | Baseline, Mid-point |
| Age, marital status, religious preference, occupational status, and education level | Demographic Form | Baseline |
| Stage of cancer, comorbid conditions, T-stage, Gleason score, type and duration of hormone therapy, the planed number of RT fractions, number of RT fields | Information on co-morbid conditions and medications was obtained from the partici-pant's interview, and corroborated using the health record. The remaining Health Form information was | Baseline |

| Variables | Method of Measurement | Frequency |
|---|---|---|
| | obtained from their health record. | |
| Infections, PSA, CBC, albumin, thyroxine, | Health Form information gathered from their health record. | Baseline |
| BMI | Obtained by the PI | Baseline, Mid-point, and End of Treatment |
| Energy Expenditure | International Physical Activity Questionnaire Short Form (IPAQ-SF) | Baseline, Mid-point, and End of Treatment |
| Sleep Disturbance | PROMIS-Sleep disturbance (PROMIS-SD) | Baseline, Mid-point, and End of Treatment |
| Depression | Hamilton Depression Rating Scale (HDRS) | Baseline, Mid-point, and End of Treatment |

**Cancer Related Fatigue (CRF; see Appendices I & J).** The subjective perception of CRF is defined operationally for this study as the score from the Spanish-validated version of the Fatigue sub-scale from the Functional Assessment of Cancer Therapy- Fatigue QOL questionnaire, the FACT-F. The instrument was developed by Cella and colleagues (1997)[108] specifically for cancer survivors.[108-109] The FACT-F 13 statements about fatigue (e.g. "I feel weak all over", "I am too tired to eat") were rated by the patients who were asked to indicate the degree to which they felt that each statement was true during the preceding week. Each item is anchored by a five-point Likert-type scale response (0 = not at all, 1 = a little bit, 2 = somewhat, 3 = quite a bit, or 4 = very much). Scores on the FACT-F can range between zero and 52. After appropriately reverse coding items 7 and 8, scoring for this scale was computed by adding the individual item scores, and dividing by the number of items answered. Higher scores represent less severe fatigue.[109-110]

The FACT-F has been rigorously tested for reliability and validity. Recently, reliability and validity testing of the scale was conducted on 131 mixed-diagnosis cancer patients participating in a longitudinal observational study of fatigue and quality of life during chemotherapy.[111] The FACT-F showed strong internal consistency (Cronbach's alpha = .93 to .95). Further, fatigue was assessed twice: at baseline and three to seven days later to evaluate test stability (test–retest reliability) that reflected good test stability over time (intraclass $r_{FS}$ = 0.89). The FACT-F was found to successfully discriminate patients based on hemoglobin (Hb) level ($r = 0.75$ $p < 0.001$). In the original reliability and validity testing of the scale, using 49 cancer patients during treatment, validity testing revealed: a significantly positive relationship with other known measures of fatigue (Piper Fatigue Scale; $r = 0.77$; POMS Fatigue, $r = 0.83$); a significant negative relationship with vigor (Vigor subscales of the Profile of Mood States; $r = -0.61$); and an anticipated lack of relationship with social desirability (short form of the Marlowe-Crowne Social Desirability Scale; $r = 0.07$).[108-110]

The FACT-F has been validated with Spanish-speaking cancer patients, with good psychometric properties including: a significantly positive relationship with other known Spanish measures of fatigue (Perform Questionnaire; $r = 0.80$, $n = 437$; POMS Fatigue, $r = 0.64$, $n = 92$); overall Cronbach's alpha of 0.89.[72,112] Researchers concluded that: "Spanish language translation as reported here provides sufficient assurance of equivalence to the English-language version to proceed with its use in clinical trials and clinical practice (p. 1,417)."[72]

The FACT-F has been widely used in clinical studies for fatigue interventions[113] including breast cancer survivors' responses to treatments. For example, in a randomized controlled trial of an individual-based intervention that combined social support and physical activity, Naumann et al.[113]found a significant reduction in fatigue among Australian women when compared with a counseling only group using the FACT-F instrument. In addition, Saligan and Kim[15] found that "the FACT-F is the most preferred instrument to measure CRF because it has been used extensively in large studies, has been shown to be sensitive to clinically significant changes in fatigue, and has robust psychometric properties" (p. 2).[15]

**Gene expression of peripheral blood cells.** Peripheral blood samples (2.5 mL) were collected from each subject at baseline and at mid-point using PAXGene blood RNA tubes (Qiagen, Frederick, Maryland). The collection tubes with peripheral blood cells were inverted 10 times to ensure red blood cell lysis immediately after collection. Although the PAXGene blood RNA tubes can be kept up to three days at room temperature (15–25° C) prior to storage in a –80° C freezer, the samples were kept at room temperature in a safe transportation box until stored within four hours in a -80° C freezer at the Puerto Rico Clinical Research Center until RNA extraction. According to the manufacturer, the advantage of this tube is that the tube contains an additive that stabilizes the in vivo gene transcription profile by reducing in vitro RNA degradation and minimizes gene induction. One study showed that this tube reduced RNA degradation compared with whole blood collected in tubes containing anticoagulants like EDTA extracted by an organic method.[40]

Prior to RNA extraction, the PAXgene Blood RNA tubes were placed out of the freezer overnight at room temperature (15-25$^0$C) in order to achieve complete lysis of blood cells. The bench areas were clean with RNase-free water to prevent contamination of the blood samples. The process of RNA extraction and purification were as follows: (a) centrifugation to pellet nucleic acids in the Paxgene Blood RNA tubes: the PAXgene Blood RNA tubes were centrifuge for 10 minutes at 3500xg and at 22$^0$C using a swing-out rotor to prevent tubes from breaking during centrifugation; (b) the supernatant was removed by decanting and the pellet of lymphocytes was retained; (c) wash pellet: 4 ml RNase-free water was added to the pellet; (d) re-suspend the pellet: the tube was vortexed until the pellet was visibly dissolved (assuring to keep the pellet), followed by centrifuging the tube for 10 minutes at 3500xg and at 22ºC; (e) using a pipette (by decanting), the supernatant was once again removed and discarded; (f) 350ul buffer 1 was added and vortexed until the pellet was visibly disolved; (g) the pellet was transferred to a microcentrifuge tube: the sample was pipetted into a 1.5 ml microcentrifuge tube; Manual RNA purification followed; (h) the re-suspended pellet was incubated in optimized buffers together with proteinase K to bring about protein digestion; adding proteinase K and binding buffer: 300 µl Buffer BR2 and of 40 µl proteinase K was added to the 1.5 ml microcentrifuge tube containing dissolved pellet then mixed by vortexing for 5 seconds and incubating for 10 minutes at 55° C using a shaker–incubator at 1100 rpm; (i) an additional centrifugation through the PAXgene Shredder spin column was carried out to homogenize the cell lysate and remove residual cell debris; the lysate was pipetted directly into a PAXgene Shredder spin column (lilac) placed in a 2 ml processing tube, and then centrifuged for 3 minutes (19900 xg); (j) the supernatant of the flow-through fraction was transferred to a microcentrifuge tube and ethanol was added to adjust binding condition: the entire supernatant was transferred to a new 1.5 ml microcentrifuge tube without disturbing the pellet in the processing tube and 350 µl ethanol (96–100%) was added, next it was mixed by vortexing and centrifuging briefly (1–2 seconds at 500–1000 x g) to remove drops from the inside of the tube lid; (k) during a brief centrifugation, RNA is selectively bound to the PAXgene silica membrane as contaminants pass through: 700 µl sample was pipetted into the PAXgene RNA spin column (pink) placed in a 2 ml processing tube, and centrifuged for 1 minute at 8000–20,000 x g (15,500 xg); then placing spin column in a new 2ml processing tube, discarding old processing tube containing the flow-through and repeat steps with remaining sample (l) remaining contaminants are removed in several wash steps: 350 µl Buffer BR3 was pipetted into the PAXgene RNA spin column, centrifuged for 1 minute at 8000–20,000 x g (15,500 xg) and the spin column was placed in a new 2 ml processing tube, the old processing tube containing flow-through was discarded; (m) the membrane is treated with DNase to remove traces amounts of bound DNA ( DNA digestion to ensure elimination of genomic DNA): 10 µl DNase I stock solution was added to a 70 µl Buffer RDD in a 1.5 ml microcentrifuge tube and mixed; the DNase I incubation mix (80 µl) was pipetted onto the PAXgene RNA spin column membrane, and placed on the benchtop (20–30°C) for 15 minutes; (n) washing: 350 µl Buffer BR3 was pipetted into the PAXgene RNA spin column, and centrifuged for 1 minute at 8000–20,000 x g (15,500 xg) followed by placing the spin column in a new 2 ml processing tube and discarding the old processing tube containing flow-through; 500 µl Buffer BR4 was pipetted to the PAXgene RNA spin column, and centrifuging for 1 minute at 8000–20,000 x g (15,500 xg) followed by placing the spin column in a new 2 ml processing tube and discarding the old processing tube containing flow-through; another 500 µl Buffer BR4 was added to the PAXgene RNA spin column and centrifuged for 3 minutes at 8000–20,000 xg (15,500 xg); (o) the tube containing the flow-through was discarded and the PAXgene RNA spin column was placed in a new 2 ml processing tube followed by Centrifuge for 1 minute at 8000–20,000 xg (15,500 xg); (p) after the wash steps, RNA is eluted in elution buffer (Buffer 5) and heat-denatured; elution in the tube containing the flow-through was discarded , then the PAXgene RNA spin column was placed in a 1.5 ml microcentrifuge tube, and 40 µl Buffer BR5 was pipetted directly onto the PAXgene RNA spin column membrane followed by centrifugation for 1 minute at 8000–20,000 x g (15,500 xg) to elute the RNA and this step was repeated; the eluate was incubated for 5 minutes at 65°C in the shaker–incubator without shaking followed by chilling immediately on ice (This incubation at 65°C denatures the RNA for downstream applications). The quantity of total RNA was measured by a spectrophotometer at optical density of 260 nanometers. RNA quality was assessed using the RNA 6000 NanoLabChip® on a Bioanalyzer Agilent 2100 (Agilent Technologies, Palo Alto, CA); (q) lastly, the RNA samples were stored at a -80° C freezer. The above described method (RNA extraction) was conducted at the laboratory of Dr. Leorey N. Saligan, PhD, CRNP, RN, Symptom Biology Unit, National Institute of Nursing Research (NINR), Intramural Research Program, National Institutes of Health, Bethesda, MD. All biologic samples were coded and stored in a locked -80° C freezer at the UPR Clinical Research Center facilities until a monthly shipment via FEDEX to the NINR for analysis. The monthly shipment of blood samples followed the manufacturer's procedure and the standard operating procedures of the Puerto Rico Clinical Research Center approved by the UPR Institutional Review Board and Biosafety Committees.

**Demographic form (see Appendices G & H).** Demographics included the respondent's age, marital status, religious preference, occupational status, and education level. The PI obtained that information from the participants' self-report on the demographic form.

**Health form (see Appendix P).** The PI obtained the health information for this form from the participants and from their RT medical chart. The PI asked the participants about co-morbid conditions (e.g. HTN, Diabetes) and current medications. In general, the participants' knowledge about their current medications was limited, therefore, the PI used the health record to clarify the information. The clinical information obtained from the medical chart included: stage of cancer, Gleason score, type and duration of hormone therapy, number of RT fractions and number of RT fields. Laboratory results including PSA level, CBC, albumin, thyroxine were only available at baseline visit, however laboratory results other than PSA level were not available for some participants. Histories of infections, visits to ER, hospital admissions or need for interruption of treatments were recorded at each visit. Weight and height measurements obtained by the PI were recorded on this form.

**Body Mass Index (BMI).** Weight was measured once at each time point using a manual scale. Participants were instructed to stand straight with no shoes and to wear light clothes. Weight was recorded in kilograms (kg) and height in centimeters (cm) and later converted to meters. For this study, BMI was the person's weight (in kilograms) divided by the square of their height (in meters).[114] While BMI is the most commonly used measure for estimating whether an individual person is overweight or obese, it is also an indicator of excess body fat. Overweight or BMI $\geq 25$ kg/m$^2$ has been found to be highly correlated with clinically significant fatigue nine months after breast cancer treatment.[115] Therefore, BMI has commonly been included as a potential confounder in longitudinal studies of fatigue.

**Energy expenditure (see Appendices K & L).** Energy Expenditure was defined operationally as the score on the International Physical Activity Questionnaire Short Form (IPAQ-SF). The IPAQ was developed in 1998 by the International Consensus Group for Physical Activity Measurement (1996), with short and long versions of the questionnaire.[116] The purpose of the Consensus group was to develop a self-reported measure of physical activity suitable for assessing population levels of physical activity across countries. The IPAQ-SF self-administered format consisted of nine items that ask about a seven-day recall of the amount of minutes spent in activity at four intensity levels: (a) vigorous-intensity activity such as aerobics; (b) moderate-intensity activity such as leisure cycling; (c) walking; and (d) sitting. It includes items such as "During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?" For all categories respondents had to specify how many days and how many minutes each day they spent on a specific activity category.

Meus et al.[117]summarize the guidelines for data processing and analysis of the IPAQ-SF. First, for all categories, the amount of Metabolic Equivalents (METs)-minutes was calculated by multiplying the number of minutes by 8.0 (vigorous), 4.0 (moderate), 3.3 (walking), or 1.3 (sitting). Second, a total score was calculated by adding the METs-minutes of the first three categories together. Lastly, in order to obtain the energy expenditure (expressed in kilocalories or kcal), METs-minutes were multiplied by the respondent's weight in kilograms and then divided by 60 (METs-minutes x kg weight/60).

Craig et al.[118] reported a reliability and validity study of the IPAQ-SF that were completed in 14 centers in 12 countries using healthy adults. Results showed a good test-retest-reliability (Spearman's $\rho$ = 0.80) and a moderate criterion validity (Spearman's $\rho$ = 0.30) with an accelerometer. Recently, similar results were reported by Ramırez-Marrero et al.[119]during the validity and reliability testing of the Spanish version of the IPAQ-SF using 58 Hispanic Puerto Ricans living with HIV. The IPAQ-SF Spanish version was administered by personal interview before and after the 7-day evaluation period with the ActiGraph and DigiWalker. Test-retest reliability was acceptable: IPAQ measure one was significantly ($p < 0.05$) related to the IPAQ measure two with $r$ values ranging from 0.32–0.75; and, Spearman correlation coefficients between the sleep /sit (min/d) IPAQ 2 nd measure and ActiGraph were only modestly correlated ($r_s = .28$, $p = .05$). However, the IPAQ min/day of moderate Physical Activity correlated well with the Digi Walker average number of steps/day ($r_s = .76$, $p = .04$).The IPAQ-SF is a widely used measure of physical activity, inactivity, and energy expenditure in cancer patients during treatments.[120-122] The IPAQ was used in this study instead of objective measures of physical activity (e.g., accelerometer) even though the latter may be more reliable and valid, given the high costs of acquisition and data analysis with the use of accelerometers and pedometers during longitudinal studies, and that data on physical activity was not the central concern for this study, but rather was desired for possible use as a covariate given prior research on the role of physical activity in fatigue.

**Sleep disturbance (see Appendices M & N).** The subjective experience of Sleep Disturbance was assessed by the adult PROMIS-Sleep disturbance Spanish form.[123] The adult PROMIS-Sleep disturbance short form assessed sleep disturbance (i.e., perceptions of sleep quality, sleep depth apρnd restoration associated with sleep; perceived difficulties and concerns with getting to sleep or staying asleep; and perceptions of the adequacy of and satisfaction with sleep) over the previous seven days. For example, one of the items reads: "In the past seven days my sleep was refreshing". Each item was anchored by a five-point Likert-type scale response (1 = not at all, 2 = a little bit, 3 = somewhat, 4 = quite a bit, or 5 = very much). Scores were totaled and divided by the number of items answered, to compute the mean score. A conversion table available in the manual was then used to translate this score into a T-score for each participant. The T-score re-scales the raw score into a standardized score with a mean of 50 and a standard deviation (SD) of 10. Therefore, a person with a T-score of 40 is one SD below the mean, representing less sleep disturbance that the average. The standardized T-score was reported as the final score for each participant. In testing the PROMIS with a general population sample, preliminary reliability and validity evidence showed that the short form correlated strongly with the full form, $r = 0.96$ ($n = 2{,}252$; 10% Latino). Construct validity for the sleep-disturbance was supported by a high correlation with the Pittsburgh Sleep Quality Index, $r = 0.80$ ($n = 300$) and a lower correlation with the Epworth Sleepiness Scale, $r = 0.45$. This was not surprising because sleepiness is a slightly different construct from sleep disturbance.[124]

**Depression (see Appendix O).** The Hamilton Depression Rating Scale (HDRS) is a clinician-rated paper questionnaire developed by Hamilton in 1967 for psychiatry in-patient and out-patient settings.[125-126] While initially intended to be used by a psychiatrist, studies have shown that it can be used by clinically inexperienced researchers after standardized training.[127] Bagby's[128] review of literature in 2004 on 70 studies that examined the psychometric properties of the HDRS scale showed overall good psychometric properties such as: internal reliability ranging from Cronbach's alpha ($\alpha$) of 0.46 to 0.97; inter-rater reliabilities (Pearson's $r$) ranged from 0.82 to 0.98; and retest reliability (Pearson's $r$) ranged from 0.81 to 0.98. Validity of the HDRS has been reported to range from $r = 0.48$-$0.85$ with the global measure of depression, the "Beck Depression Inventory**.**" In addition, the HDRS has been employed successfully in psychopharmacological and clinical research with good internal reliability, Cronbach α's ranging from 0.81-0.98.[129]

The HDRS is composed of 17 items which are rated on a three-point (0-absent, 1-slight or trivial, 2-clearly present) or four-point (symptom is: 0-absent, 1-mild, 2-moderate, or 3-severe) scale according to standardized descriptors.[125-126] The total score can range from zero to 54. In general, the higher the total scores the more severe the depression. According to Cusin, Yang, Yeung, and Fava,[130] it is accepted by most clinicians that scores between zero and six do not indicate the presence of depression, scores between seven and 17 indicate mild depression, scores between 18 and 24 indicate moderate depression, and scores over 24 indicate severe depression. The pre-defined cutoff score for depression is 15 in cancer patients, with higher scores indicating more symptoms of depression.[129]

The HDRS has been validated in the Spanish-speaking population with good psychometric properties, including appropriate discriminative validity (HDRS-Clinical Global Impression: $p < 0.0001$), internal consistency (Cronbach's $\alpha > 0.70$), test-retest reliability (intra-class correlation coefficient [*ICC*] $\geq$ 0.9), and sensitivity to change (effect size > 1.5).[131]

## Data Analyses

**Specific Aim 1: To describe the trajectory of fatigue over the course of EBRT among Hispanic PR men receiving EBRT for non-metastatic PC and compare it with historical data of fatigue symptoms of Caucasian men with PC during EBRT.**

**Overview of approach in Aim 1.** FACT-F was administered at three time points: baseline (prior to EBRT), midpoint (days 19-21), and completion (days 38-42) of EBRT. FACT-F scores obtained from this study were compared with the published FACT-F scores for the general US population and from scores for Caucasian cancer patients.

**Statistical analysis.** A code book was created prior to data entry. All the data obtained from paper measures were double entered in a Microsoft Excel data base format developed by the data manager of the Cancer Center and coded and locked in a secured location. Descriptive statistics were generated for the participants' demographic and clinical characteristics as well as for the FACT-F, PROMIS-SD, and HDRS scales and for the IPAQ for each of the time points.

To assess the change in fatigue at the three time-points (pre, midpoint, and end of EBRT), we compared all pair-wise fatigue scores measured at the three time points (i.e., pre vs midpoint; pre vs end, midpoint vs end) using both parametric (paired t-tests) and non-parametric (Wilcoxon Signed Rank Test) tests to assess the robustness of results. In addition, we conducted subset analysis and case levels of the fatigue scores using descriptive statistics. The change in energy expenditure, sleep disturbance and depression at the three time-points (pre, midpoint, and end of EBRT) was assessed using paired t-tests. We also assessed for significant differences between those on ADT and those not on ADT in the changes in fatigue (baseline minus midpoint), using independent sample t-test. Finally, multiple linear regressions (baseline, mid-point and end-point) were conducted to assess the impact of several independent variables on the dependent variable of fatigue.

**Power analysis.** We planned a study of a continuous response variable, fatigue, collected at multiple time-points for each study subject. Assuming the change in fatigue score between two measurements was normally distributed, we estimated that the standard deviation in the change of fatigue score was around 10. This estimate was based on the following: the anticipated range = maximum change – minimum change, observed to be around 40, yielding an estimated $SD \approx 10$ based on empirical rule for normal distribution. The empirical rule states that approximately 95% of the differences will be within two *SD* of the mean. Therefore, a power analysis for a desired power of .8 and $\alpha = .05$, suggests the need for 26 subjects to detect a mean score change of five points. The Type I error probability associated with this test of this null hypothesis is 0.05 for a one-sided test that fatigue gets worse over the course of EBRT. Power calculations were performed using R 2.15.0 for Windows.

**Pitfalls and resolution.** It is an acknowledged limitation that the current study is based on a self-report measure of fatigue. However, symptoms by definition are subjective and self-report measures of CRF are in fact, most appropriate and can provide the strongest evidence of support for behavioral interventions, particularly when instruments with good psychometric properties have been culturally validated and adapted for the population to be studied. Although several important control variables were included in the study and controlled for in the association testing, due to the relatively small sample size of this study we were unable to control for all potential variables that might affects CRF among PR prostate cancer patients during EBRT (e.g., anxiety).

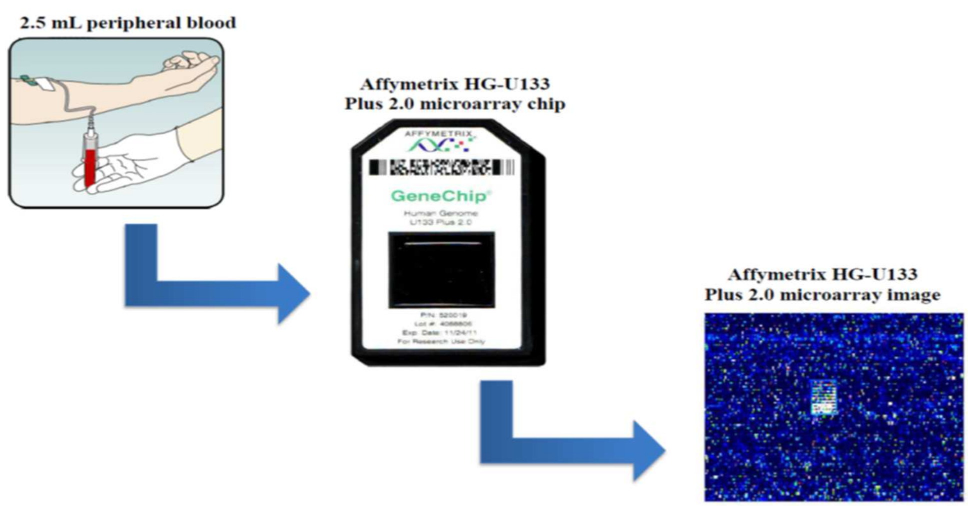
**<u>Specific Aim 2</u>: To assess gene expression changes from baseline to midpoint of EBRT among Hispanic PR men receiving EBRT for non-metastatic PC.**

**Overview of approach in Aim 2.** In order to capture the initial inflammatory response of EBRT which peaks at midpoint (day 21), only levels of the differentially expressed genes at baseline and at midpoint of EBRT were assessed. Hence, for this aim involving genome-wide assessment of gene expression levels we focused on determining the changes in expression of genes from whole blood samples that were collected at baseline and midpoint of EBRT. A total of 2.5 mL of peripheral blood was collected using RNA PAXGene tubes (Qiagen Frederick, MD) for each of the two study time points. All biologic samples were stored in a securely-locked -80$^{o}$C freezer until shipment to Dr. Saligan's laboratory at the NINR for RNA extraction. De-identified blood samples were transferred via FEDEX to NINR for analysis. De-identified blood samples microarray data results were returned via secure file from NINR for analysis.

**Gene expression microarray.** RNA extraction, purification, cDNA and cRNA synthesis, amplification, hybridization, scanning and data analyses were conducted at the NINR lab by the same technician following standard protocols. After total RNA isolation and extraction (described on p. 56) from frozen whole-blood samples following the PAXgene blood RNA kit procedure (PreAnalytiX). RNA yields were 300 ng or greater from each 2.5 mL of whole blood collected. The quantity of total RNA was measured by a spectrophotometer at optical density of 260 nanometers. RNA quality was assessed using the RNA 6000 NanoLabChip® on a

Bioanalyzer Agilent 2100 (Agilent Technologies, Palo Alto, CA). All extracted RNA was purified using RNeasymini kit (Qiagen, Valencia, CA). The total RNA concentration was tested using the NanoDrop (ND-1000; Wilmington, DE), and the purity and integrity with the Experion systems (Biorad, Hercules, CA). Following RNA preparation, the samples were treated with DNase to ensure elimination of genomic DNA. A total of 100 to 150 ng of extracted RNA per sample was then converted to cDNA using the RT2 First Strand Kit (SABiosciences, Frederick, MD). After cDNA synthesis reaction, the cDNA was diluted using nuclease-free $H_2O$ and immediately stored at -20$^{o}$ C until used for human gene expression profiling. Affymetrix microarray chips (HG U133 Plus 2.0, Santa Clara, CA) were used to assess gene expression levels for genes across the genome. The Affymetrix HG-U133 Plus 2.0 microarray chip is composed of more than 54,000 probe sets and 1,300,000 distinct oligonucleotide features, which can analyze the expression level of over 47,000 transcripts, including 38,500 well characterized human genes. Affymetrix Gene Chip Command Console (AGCC, 3.0 V) was used to scan the images for data acquisition (i.e., gene expression level).

**Figure 6. Summary of the Major Steps for the Microarray Experiments**




Provided by Dr. Leroy N. Saligan

Figure 6 summarizes the process involved in the microarray experiments. The first square shows the process of peripheral blood collection using RNA PAXGene tubes. After RNA extraction and purification, 50ng of total RNA were required for global expression profiling. Prior to hybridization on arrays, RNA samples were amplified, fragmented and labeled with biotin. Square 2 shows that after amplification, RNA was combined with hybridization cocktails and transferred to Affymetrix arrays and incubated for 18 hrs at 37° C in a hybridization oven. After hybridizing samples onto the probe array, the chips were washed and stained. Square 3 shows that after staining, the chips were scanned; then, the scanning software converted raw image data (.DAT files) to numeric intensity files (.CEL).

**Statistical analysis.** Affrymetrix CEL files (the file format that stores the results of the intensity calculations of the pixel values of the scanned image from a microarray chip) were imported into *LIMMA*[132] package in R. Background correction, quantile normalization, and summarization were conducted with the Robust Multichip Average (RMA) algorithm developed by Irrizarry.[133] RMA consists of three steps: (a) background correction: a preprocessing step employed to correct for background noise and processing effects in the microarray data in which probe-level data for each microarray are background corrected independently using a probabilistic model; (b) quantile normalization: whereby the background corrected probe-level data on each microarray were normalized to a common set of quantiles, derived from background corrected data from all microarrays; and (c) summarization: which computes an expression value for each gene from all the probes for the gene. Then, assessment of differentially expressed genes between time-points was assessed using the *LIMMA* package. This step consists of the *LIMMA* program fitting for each gene a linear regression model to determine if genes were differentially expressed using a modified $t$-test (to find differences between midpoint and baseline expression) that shrinks non-differentially expressed gene effect sizes towards zero (or fold-change towards 1). The fold-change is initially calculated as an antilog of a mean log fold-change over all possible between-chip comparisons contributing to the midpoint vs. baseline comparison. Both a p-value and a Benjamini-Hochberg false discovery rate (FDR) were computed to adjust for multiple testing.[132,133] The FDR is the proportion of discoveries that are false among all discoveries, i.e., the proportion of incorrect rejections among all rejections of the null hypothesis. Subsequently, a *p*-value adjustment was conducted using the *LIMMA* global method with the Benjamini-Hochberg approach for control of false discovery rate (FDR). The FDR is the proportion of discoveries that are false among all discoveries, i.e., the proportion of incorrect rejections among all rejections of the null hypothesis. In order to restrict the false discovery rate to 0.01, all the genes with a FDR less than 0.01 were considered candidate differentially-expressed genes. Lastly, the genes from the list of the FDR <0.01 were then sorted and filtered by the log fold change column to obtain a list of the top 10 significant up-regulated genes, and the top 10 significant down-regulated genes.

In order to obtain a better understanding of the physiologic pathways that are associated with the genes that are differentially expressed from baseline to midpoint of EBRT, a functional network analysis was conducted. Ingenuity Pathway analysis (Ingenuity® Systems,www.ingenuity.com, Redwood City, CA) identified functional networks of the differentially expressed genes from the Ingenuity's Knowledge Base. Functional networks and the top differentially expressed genes that were identified from baseline to midpoint of EBRT in this study were compared for similarities with the functional networks and top differentially expressed genes identified from Caucasian men during EBRT reported by Saligan et al.[15-16,19]

These functional networks suggested biological underpinnings of the possible physiological mechanisms that influence fatigue intensification during EBRT in this population.

**Power Analysis.** With 26 subjects included in the study (the minimum sample size needed to meet aim 1), we had 80% power to detect a minimum of 2.0 fold change in gene expression from baseline to midpoint of EBRT. Power was computed using the web application at http://bioinformatics.mdanderson.org/MicroarraySampleSize/. For 80% power, the required sample sizes (number of subjects with paired baseline-midpoint measurements) for various fold changes, number of expressed genes analyzed and number of false positives that are acceptable are presented in **Table 2.**

Table 2

*Sample size needed to detect differentially expressed genes between baseline and midpoint of EBRT.*

| Sample Size | #Expressed Genes | # of Acceptable False Positives | Fold Change |
|---|---|---|---|
| 19 | 10,000 | 5 | 2 |
| 21 | 10,000 | 2 | 2 |
| 21 | 5,000 | 1 | 2 |
| 22 | 10,000 | 1 | 2 |
| 23 | 15,000 | 1 | 2 |
| 24 | 20,000 | 1 | 2 |

**Pitfalls and resolution.** One could argue that using blood to assess gene expression patterns may not be optimal when compared to gene expression measured in the tissue of interest (i.e., tumor tissue). However, since the etiology of fatigue remains unknown and no specific system has been directly linked to its development, a tissue-specific approach is not an ideal path to pursue at this time. Early studies using lymphocytes and RNA extracted from these cells for gene expression analysis have demonstrated that this approach is sensitive for the detection of patterns of gene expression in association with a variety of medical conditions.[17,47, 80] We did not have any technical difficulties in the blood sample collection and conservation of RNA until extraction since the process of collection, transportation, storage, and shipment of blood samples followed the manufacturer's procedure and the standard operating procedures of the UPR Cancer Center approved by the UPR Institutional Review Board and Biosafety Committees.

**Specific Aim 3: To determine the association between changes in genes expression with changes in fatigue score from baseline to midpoint of EBRT in Hispanic PR men with non-metastatic PC.**

**Overview of approach in Aim 3.** Three hundred seventy-three genes (130 up-regulated and 243 down-regulated) were differentially expressed from baseline to midpoint of EBRT after the genes passed filtering criteria of 1% FDR (see Appendix R). The changes in expression level of these genes were going to be associated with changes in fatigue scores from baseline to midpoint of EBRT. The purpose was to determine not only which genes are differentially expressed pre and at midpoint EBRT, but also those genes where the change is associated with a change in fatigue (i.e., EBRT associated genes that are also associated with changes in level of fatigue). Due to the surprising finding that fatigue did not significantly change over the course of EBRT (see Chapter 4), the planned analyses for Aim 3 were not possible.

## Ethical considerations

Human Research Protection Office's approval was obtained from the University of Puerto Rico and the U.S. Midwestern academic medical center Human Subjects Committees. No participants experienced psychological distress from answering the fatigue, IPAQ, PROMIS-sleep disturbance questionnaires and, HDRS nor experienced any bruise or sign of infection from the venipuncture site at any time-point. To safeguard confidentiality, unique identifiers were assigned to all participants for all portions of the study and all data collection instruments and blood samples. Logs linking participants' identifying information to study numbers are kept locked in a file cabinet at the UPR School of Nursing, available only to the PI and her mentoring team. Names of study participants will not be reported at any time; only the data obtained as a result of their participation will be made public; study findings will be presented only in the aggregate.

All responses from paper questionnaires collected at all study time points are kept in a secure cabinet at the PI's office in the UPR School of Nursing. All biologic samples were coded and stored in a locked -80$^{o}$ C freezer at the UPR Clinical research Center facilities until shipped to the laboratory of Dr. Leorey N. Saligan, PhD, CRNP, RN, Symptom Biology Unit, National Institute of Nursing Research, Intramural Research Program, National Institutes of Health, Bethesda, MD. Every effort was made to ensure participant confidentiality as prescribed by U.S. Midwestern academic medical center Human Subjects Committees and UPR Medical sciences Campus Institutional Review boards and HIPAA standards.

**Potential risks.** We acknowledge that there were a small number of relatively minor risks associated with participation in the study. Even though the participants were given the option to withdraw from the study at any time during its course, no one withdrew. In the rare event a participant experienced severe distress, or if the score on the HDRS scale was above the cut-off score for depression, or if participants reported signs of infection around the venipuncture site, they would have been referred for evaluation, counseling, and follow-up medical care services. However, during the study there were no side effects, hence no patient evaluation was needed. We did not offer any participation incentives.

# CHAPTER IV

# RESULTS

The purpose of this study was to examine the clinical fatigue experienced by 26 Hispanic Puerto Rican men over the course of external beam radiation therapy (EBRT). Also, an unbiased a microarray platform was used to explore the differential expression of genes from peripheral whole blood RNA collected from Hispanic Puerto Rican men at baseline and at midpoint of EBRT. Functional networks of the differentially expressed genes were examined using Ingenuity Pathway Analysis to determine pathways that may explain the possible physiological mechanisms that influence fatigue intensification during EBRT in this population. A comprehensive database that included demographics and study measures responses at three time points (baseline [prior to EBRT], midpoint [days 19-21], and end of treatment [days 38-42]) from the 26 participants was developed. An independent reviewer examined the data entry and confirmed that each data point was accurately entered. The medical record and the participant-reported data were analyzed using SPSS 18.0 statistical software, and differential expression of genes data were analyzed using the *LIMMA*[132] package in R. This chapter presents the results of the study, including the participants' characteristics and the findings for each study aim.

## Sample

Thirty-one individuals were approached for possible participation in the study; three refused participation, 28 agreed, and 26 of these were eligible and gave consent. The reasons for those that refused to participate were: "agreed to answer questionnaires but not to give blood" (2 patients) and "not interested" (1 patient). Of the 28 who agreed to participate, two participants were found to be ineligible due to a diagnosis of chronic renal failure, and that they were currently on dialysis. As no one dropped out of the study, data were obtained from the required number of subjects identified in the power analysis. To avoid missing data points from the instruments administered, prior to the participants leaving the site, the principal investigator reviewed that the participants answered all the instruments' items and kindly requested participants to complete any missing items. Data were missing on some laboratory results from the radiotherapy medical chart: 19 had thyroid-stimulating hormone (TSH) levels missing; 10 had albumin levels missing, and four had hemoglobin levels missing. However, the medical history and the inclusion/exclusion criteria interview confirmed no disease exclusion and since these participants had no values missing on each instrument, they were all included in the data analyses. Blood samples were taken from all the participants at baseline and midpoint of EBRT for the gene expression analyses. This resulted in a sample of 26 participants who completed all the study procedures.

**Sample Demographics**

The convenience sample consisted of 26 Hispanic Puerto Rican men, ages 52-81 years, all being treated with EBRT for non-metastatic PC at one site ("Tome" ambulatory Radio Oncology Center). The average age was 67.01, with a *SD* of 7.56. The majority of participants reported falling in the racial category of White (85%). Most of the participants were Catholic (73.1%), and retired (69.2%). While 96.2% of the participants were married or partnered, three (11.5%) participants reported that they cared for themselves on their own or were caring for their dementia-affected spouse. Participants' number of children ranged from zero to six, with a mean of three (*SD* = 2). The participants were for the most part well educated with only four (15.4%) participants not having a high school diploma. Fifty percent of the participants had a baccalaureate degree or higher education. A summary of the sample demographics is provided in Table 3.

Table 3

*Demographic Characteristics of Study Participants*

| **Characteristics** | ***N*** | ***(%)*** |
|---|---|---|
| Racial categories | | |
| White | 22 | (85.0) |
| African American | 4 | (15.0) |
| Marital status | | |
| Married | 24 | (92.3) |
| Single | 1 | (3.8) |
| Living together | 1 | (3.8) |
| Highest education completed | | |
| Elementary/middle school | 2 | (7.7) |
| Some High School | 2 | (7.7) |
| High School Diploma | 8 | (30.8) |
| Some university no degree | 1 | (3.8) |
| Bachelor | 7 | (26.9) |
| Doctoral degree | 3 | (11.5) |
| Post-doctoral | 3 | (11.5) |
| Religious preferences | | |
| Catholic | 19 | (73.1) |
| Protestant | 3 | (11.5) |
| None | 4 | (15.4) |
| Occupation | | |
| Retired | 18 | (69.3) |
| Working | 7 | (26.9) |
| Handicap | 1 | (3.8) |
| Primary caregiver | | |
| Wife | 23 | (88.5) |
| None | 3 | (11.5) |

**Sample Clinical Characteristics**

Approximately one third of the sample (34.6%) had low risk disease with a prostate cancer clinical stage of T1 and a Gleason score between six and nine, 46.2% had stage T2, 15.4% had stage T3, and one participant had stage T4 prostate cancer. Baseline prostate-specific-antigen levels ranged from 0.02 to 17.70ng/mL. Most participants (57.7%) received neo-adjuvant hormonal therapy (androgen deprivation therapy) eight weeks prior to the initiation of EBRT, and only 23.1% received radical prostatectomy more than a year before receiving EBRT. Of the 26 participants, 14 (53%) received a total of 43 fractions with 77.4 Gy, six (23%) received a total of 42 fractions with 75.6 Gy, five (19%) received a total of 38 fractions with 68.4 Gy, and one (5%) received a total of 38 fractions with 61.2 Gy using the IMRT technique. Baseline hemoglobin (*M* = 13.96 [*SD* = 1.17]), albumin (*M* = 4.06 [*SD* = .28] g/dL), and TSH (*M* = 2.68 [*SD* = 1.33] ųIU/mL) were within reference range (see Table 4). None of the participants had uncorrected hypothyroidism, anemia, or chronic inflammatory disease. More than half of the participants had co-morbid conditions such as hypertension (69.2%) and diabetes (53.8%). The average body mass index (BMI) across the three time points was 29.39 (*SD* 4.16), consistent with being overweight.[134.] None of the clinical features in table four were significantly correlated with fatigue at baseline.

Table 4

*Participants' Clinical Characteristics during Prostate Cancer Treatment*

| Variable | Mean | SD | Range | N | Reference Range |
|---|---|---|---|---|---|
| Gleason score (median) | 7.00 | .99 | 6-9 | 26 | |
| PSA levels, ng/mL | 5.42 | 4.34 | .02-17.70 | 26 | 0-2.5 ng/mL |
| Hemoglobin | 13.96 | 1.17 | 12.20-17.70 | 22 | 11-18g/dL |
| Albumin levels, g/dL | 4.06 | .28 | 3.4-4.5 | 16 | 3.4-5.0 g/dL |
| TSH, µIU/mL | 2.68 | 1.33 | 1.18-5.27 | 7 | 0.465-4.68 µIU/mL |
| Number of RT Fractions (median) | 42.00 | 2.10 | 38-43 | | |
| Number of RT Fields (median) | 7.00 | .39 | 6-8 | | |

## Instrument Evaluation

Due to the relatively small sample size, visual inspections as well as frequency distributions of the data were conducted on all items to identify outliers and responses inconsistent with instrument options. Histograms were evaluated for normalcy of distribution. Descriptive characteristics of mean, mode, median, variance, standard deviation, kurtosis and skew were generated and reviewed. Internal consistency reliability and the interrelationship among items were investigated based on the data from all self-report instruments across the three time points. Mean scores of all scales were calculated. As previously stated, there were no missing values on each instrument.

**Internal Consistency Reliability**

Coefficient alphas were calculated for all instruments at the three time points and item analysis for each scale was conducted using the reliability analysis procedure. The *Cronbach alphas* for all the numeric rating scales at the three time points are presented in Table 5. The Functional Assessment of Cancer Therapy-Fatigue (FACT-F) and the PROMIS-Sleep disturbance (PROMIS-SD) scale showed acceptable internal reliability. The range of the Cronbach alphas for the FACT-F scale across the three time points was .91-.93, for the PROMIS-SD was .89-.95, and for the Hamilton Depression Rating Scale (HDRS) was .55-.84.

Table 5

*Cronbach Alpha Reliability of the Functional Assessment of Cancer Therapy-Fatigue (FACT-F), PROMIS-Sleep disturbance (PROMIS-SD), and the Hamilton Depression Rating Scale (HDRS) across the 3 Time Points*

| **Reliabilities of Scales Across Time Points** | | | |
|---|---|---|---|
| Scales | Baseline | Midpoint | End of treatment |
| | *Cronbach Alpha* | | |
| Fatigue (FACT-F) | .91 | .92 | .93 |
| PROMIS-total score | .92 | .89 | .95 |
| HDRS | .86 | .55 | .84 |

The HDRS is not a self-report measure; it is a clinician-rated paper questionnaire that allowed the principal investigator to assess participants for symptoms of depression. At each time point, all participants were rated zero on a number of items (at baseline, all participants were rated 0 on 6 of the 17 items, at midpoint on 7 items, and at end of treatment on 6 items). This resulted in these items having no variability. Therefore, in assessing the internal consistency reliability for the HDRS, the SPSS program excluded these items from the analysis. Table 6 show which items all participants were rated zero across the three time points. Lastly, because the International Physical Activity Questionnaire Short Form (IPAQ-SF) is not a scale, but rather provides the amount of Metabolic Equivalents (METs)-minutes of activity, no Cronbach alpha was determined for this measure.

Table 6

*Items from the Hamilton Depression Rating Scale that the Participants' Assessment was **0** across the 3 Time Points*

| **Baseline** | **Midpoint** | **End of Treatment** |
|---|---|---|
| Item # 3 | Item # 1 | Item # 2 |
| Item # 8 | Item # 3 | Item # 3 |
| Item # 10 | Item # 8 | Item # 8 |
| Item # 12 | Item # 9 | Item # 13 |
| Item # 15 | Item # 10 | Item # 14 |
| Item # 17 | Item # 15 | Item # 17 |
| | Item # 16 | |

**Note:** Specifically each item assesses: #1depressed mood, #2 feelings of guilt, # 3 suicide, # 8 psychomotor retardation, # 9 agitation, # 10 Psychic anxiety, # 12 gastrointestinal symptoms, #13 general somatic symptoms, # 14 genital symptoms, #15 hypochondriasis, # 16 loss of weight, and # 17 patient's insight for depression

**Dimensionality**

Inter-item correlations were assessed using Pearson correlation coefficients for the FACT-F, PROMIS-SD, and HDRS scales across the three time points during EBRT. With respect to the FACT-F, findings showed that: (a) at baseline, the range of inter-item correlations was from -.02 to .98; (b) at midpoint, a range from .01 to .95; and (c) at end of treatment, a range from .22 to .96. The HDRS showed: (a) at baseline, a range of inter-item correlations from -.11

to 1.0; (b) at midpoint, a range from -.20 to 1.00; and (c) at end of treatment, a range from -.09 to 1.00. Lastly, the inter-item correlation matrix for the PROMIS-SD showed that: (a) at baseline, correlations ranged from .29-.94; (b) at midpoint, from .15-.89; and (c) at end of treatment, from .5-.93. Specifically, with respect to the FACT-F: at baseline and at midpoint, seven items had inter-item-correlations above, and six items below .5, and at endpoint, 10 items had inter-item-correlations above and three items below .5. With respect to the PROMIS-SD: at baseline, seven items had inter-item-correlations above and one item below .5, at midpoint, five items had inter-item-correlations above and three items below .5, and at endpoint, all items had inter-item-correlations above .5; and with respect to the HDRS: at baseline, four items had inter-item-correlations above and seven items below .5, at midpoint, nine items had inter-item-correlations above and one item below .5, and at endpoint, eight items had inter-item-correlations above and three items below .5. In sum, the instruments in this study demonstrated overall higher, rather than lower, inter-item-correlations, and Cronbach alphas > 0.70 across the three time points, except for midpoint HDRS.

**Distributions and Mean Scores for the FACT-F, PROMIS-SD, and HDRS Scales, and the IPAQ, across the 3 Time Points**

Descriptive statistics were generated for the FACT-F, PROMIS-SD, and HDRS scales and for the IPAQ for each of the time points. For each of the three assessments (baseline, midpoint, and end of treatment of EBRT), a mean score for each scale was calculated for use in the subsequent statistical analyses. Distributions and mean scores for the FACT-F, PROMIS-SD, HDRS scales, and the IPAQ across the three time points appear in Table 7. To assess the change in sleep disturbance, energy expenditure, and depression at the three time-points (pre, midpoint, and end of EBRT), we compared all pair-wise scores measured at the three time points using paired t-tests. The paired t-test for the FACT-F will be described later under the aim 1 results.

Table 7

*Distributions and Mean Scores for the Functional Assessment of Cancer Therapy-Fatigue (FACT-F), PROMIS-Sleep disturbance (PROMIS-SD), the Hamilton Depression Rating Scale (HDRS), and the International Physical Activity Questionnaire Short Form (IPAQ-SF) across the 3 time points*

| **Scales** | ***Mean*** | ***SD*** | ***Actual range*** |
|---|---|---|---|
| FACT-F | | | |
| (possible range: 0-52) | | | |
| Baseline | 42.38 | 9.34 | 21-52 |
| Midpoint | 42.11 | 8.93 | 20-52 |
| End | 43.03 | 8.62 | 2152 |
| PROMIS-SD | | | |
| (possible range: 0-40) | | | |
| Baseline | 20.35 | 9.82 | 8-44 |
| Midpoint | 19.00 | 7.73 | 8-34 |
| End | 17.04 | 8.67 | 8-39 |
| HDRS | | | |
| (possible range: 0-54) | | | |
| Baseline | 1.23 | 2.27 | 0-9 |
| Midpoint | 1.08 | 1.41 | 0-5 |
| End | .96 | 1.93 | 0-8 |
| IPAQ-SF | | | |
| (possible range: 0 - cannot be determined*) | | | |
| Baseline | 2375.02 | 3815.64 | 0-15420.00 |
| Midpoint | 2905.65 | 4281.05 | 0-17394.00 |
| End | 3882.29 | 7056.55 | 0-29040.00 |

*Cannot be determined because there is no limit to the frequency or intensity of the exercise done.

The FACT-F is a 13-item questionnaire. After appropriately reverse coding (items 7 and 8), scoring for this scale was computed by adding the individual item scores, and dividing by the number of items answered. Higher scores represent less severe fatigue.[108-109] The mean fatigue score at baseline (pre-EBRT) was 42.38, at mid-point 42.12, and 43.04 at end of EBRT.

The PROMIS-Sleep disturbance short form assesses sleep disturbance over the past seven days. To compute the raw mean score, scores were totaled and divided by the number of items answered. In addition, the authors of the scale also present a T-score version. The T-score re-scales the raw score into a standardized score with a mean of 50 and a standard deviation (SD) of 10. A higher PROMIS raw and T-score represents more sleep disturbance. The mean sleep disturbance raw total score was 20.35 at baseline, 19.00 at midpoint of EBRT, and 17.04 at end of EBRT. The mean T-score showed a similar pattern, namely, 48.65 at baseline 47.75 at midpoint of EBRT, and 44.80 at end of EBRT. The results of the paired t-test for the PROMIS-SD, HDRS scales, and the IPAQ across the three time points appear in Table 8. As shown in Table 8 there were no significant differences in any of these variables across time.

Table 8

*Paired Samples t-test for the PROMIS-Sleep disturbance (PROMIS-SD), the Hamilton Depression Rating Scale (HDRS), and the International Physical Activity Questionnaire Short Form (IPAQ-SF) across the 3 Time Points*

| **Scales** | **Pairs** | ***t (df)*** | ***Two-sided p-values*** |
|---|---|---|---|
| PROMIS-SD | Baseline vs. Midpoint | .83 (25) | .42 |
| | Baseline vs. End | 1.80 (25) | .09 |
| | Midpoint vs. End | 1.90 (25) | .07 |
| HDRS | Baseline vs. Midpoint | .32 (25) | .75 |
| | Baseline vs. End | .60 (25) | .55 |
| | Midpoint vs. End | .43(25) | .67 |
| IPAQ-SF | Baseline vs. Midpoint | -1.23 (25) | .23 |
| | Baseline vs. | -1.63 (25) | .12 |

| Scales | Pairs | *t(df)* | *Two-sided p-value* |
|---|---|---|---|
| | End | | |
| | Midpoint vs. End | -1.06 (25) | .30 |

The HDRS comprises 17 items which are rated on a three-point (0-absent, 1-slight or trivial, 2-clearly present) or four-point (symptom is: 0-absent, 1-mild, 2-moderate, or 3-severe) scale.[125-126] A total score was obtained by adding the 17 items; the higher the total scores, the more severe the depression. The mean depression score of the HDRS was 1.23 at baseline, 1.08 at midpoint, and .96 at end of EBRT. A paired t-test showed that there were no significant changes between the baseline and midpoint HDRS total mean scores, between the midpoint and the endpoint HDRS total mean scores, or between the baseline and the endpoint HDRS total mean scores. None of the participants reached the cutoff score (15) for depression at any time point.

The IPAQ-SF self-administered format consist of nine items that ask about a seven-day recall of the amount of minutes spent in activity of four intensity level vigorous-intensity activity (1); moderate-intensity activity (2); and walking (3). For all categories respondents have to specify how many days and how many minutes they spent at a specific activity category. The amount of Metabolic Equivalents (METs)-minutes was calculated by multiplying the number of minutes by 8.0 (vigorous), 4.0 (moderate), and 3.3 (walking). A total score was calculated by counting the METs-minutes of these three categories together. The mean of a combined total physical activity score (Metabolic Equivalent of Task (MET)-minutes/week) was 2,375.02 at baseline, 2,905.65 at midpoint of EBRT, and 3,882.29 at the end of EBRT. Similar to that with the PROMIS-SD and the HDRS, paired t-tests showed that there were no significant changes between the baseline and the midpoint MET total mean score, between the midpoint and the endpoint MET total mean scores, or between the baseline and the endpoint MET total mean scores. In summary, compared with baseline, there were no significant changes in any of the scales (PROMIS-SD and HDRS) or the IPAQ-SF between baseline and midpoint, midpoint and completion, and baseline and completion of EBRT.

## Findings for Aim 1

*Aim 1: To describe the trajectory of fatigue among Hispanic Puerto Rican men over the course of receiving EBRT for non-metastatic prostate cancer and compare these findings with historical data of fatigue symptoms of Caucasian men with prostate cancer during EBRT.*

Fatigue was measured at each time point using the Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F). To assess the change in fatigue at the three time-points (pre, midpoint, and end of EBRT ), we compared all pair-wise fatigue scores measured at the three time points (i.e., pre vs midpoint; pre vs end, midpoint vs end) using both parametric (paired t-tests) and non-parametric (Wilcoxon Signed Rank Test) statistical tests to assess the robustness of results. Table 9 presents the results of the paired-t-tests and the Wilcoxon Signed Rank Tests.

Table 9

*Summary of Paired Samples t-test and Wilcoxon for the Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F)*

| **Paired Samples T-test** | | | | **Wilcoxon** | |
|---|---|---|---|---|---|
| Pairs | *Mean (SD)* | *T* | *p-value* | *Z* | *p-value* |
| Baseline vs.<br>Midpoint | 42.38 (9.34)<br>42.11 (8.93) | .179 | .859 | -.66 | .51 |
| Baseline vs.<br>End | 42.38 (9.34)<br>43.03 (8.62) | -.389 | .701 | -.53 | .60 |
| Midpoint vs.<br>End | 42.11 (8.93)<br>43.03 (8.62) | -.791 | .437 | -1.14 | .26 |

Mean fatigue score at baseline (pre-EBRT) was 42.38 for the sample. It remained about the same at mid-point 42.12, and slightly increased to 43.04 at completion of EBRT, but was not significantly statistically different. We also conducted the Friedman's test that is the nonparametric test equivalent to the repeated measures ANOVA. Those results also confirmed no significant difference (Friedman's test: $\chi^2$1.20 [*df*, 2], $p$ =.55). As a group the present sample did not change in fatigue over time.

We further compared the result of the changes in fatigue (baseline minus midpoint) among those receiving the concomitant use of neo-adjuvant hormonal therapy (ADT) ($n$ = 11) with those who did not ($n$ = 15). The t-test showed that there were no significant differences between those on ADT and those not on ADT in changes in fatigue ($t$ = -.45 (24), $p$ = 0.66); and, there was no significant correlation between changes in fatigue (baseline minus midpoint) and participant's age (*Spearman's rho* .23, $p$ = .25).

While as a group there were no s*tatistically* significant changes in fatigue over the course of EBRT, subset analysis of the fatigue scores showed that, of the 26 subjects, most of the participants also had no *clinically* significant change (i.e. > 3 point decrease) in fatigue (baseline to midpoint: 17 (65%); baseline to endpoint: 16 (62%); and, midpoint to endpoint: 18 (68.5%). As shown in Table 11, only a small number of participants had a clinically significant increase in fatigue (more than a 3-point decrease in fatigue score) across time points. Similarly, and also contributing to the lack of statistically significant difference, a small number of participants had a clinically significant *decrease* in fatigue (more than a 3-point increase in fatigue score) across time points. Table 10 presents the distribution of the participants with respect to clinically significant changes in fatigue across time points.

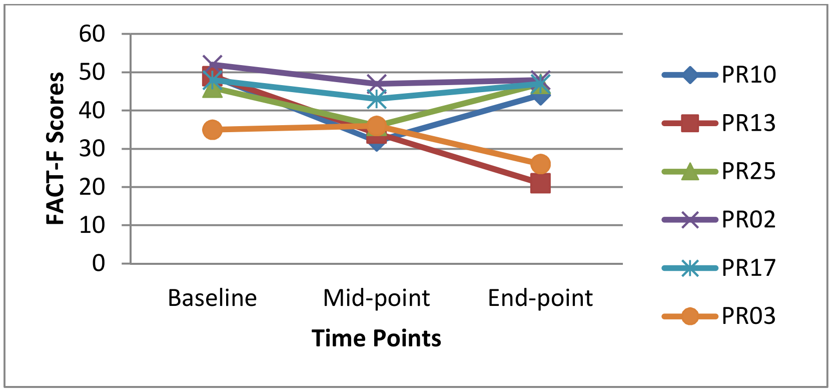
Table 10

*Distribution of the Number of Participants Reflecting Clinically Significant Changes in Fatigue across the 3 Time Points*

| **Changes in FACT-F Scores** | **Baseline to mid-point** *N (%)* | **Baseline to end-point** *N (%)* | **Mid-point to end-point** *N (%)* |
|---|---|---|---|
| more than a 3-point decrease in fatigue score (clinically significant increase in fatigue) | 5 (20.0) | 4 (15.0) | 3 (11.5) |
| more than a 3-point increase in fatigue score (clinically significant decrease in fatigue) | 4 (15.0) | 6 (23.0) | 5 (20.0) |
| 3 or less point change in any direction (no clinically significant change in fatigue) | 17 (65.0) | 16 (62.0) | 18 (68.5) |

Figure 7 shows the individual fatigue scores of those participants who had clinically significant increases in fatigue at any time point. For example, there was one participant (PR013) who not only had a clinically significant increase in fatigue from baseline to mid-point, but also from baseline to completion, and from mid-point to completion. There were three cases (PR02, PR10, PR17) who reflected a clinically significant increase in fatigue from baseline to mid-point, from baseline to completion, but remained about the same from mid-point to completion. And there was one participant (PR03) who remained about the same from baseline to midpoint, but had a clinically significant increase in fatigue from baseline to completion and from mid-point to completion.

**Figure 7. Individual Fatigue Scores of Participants Who Reflected Clinically Significant Changes in Fatigue (more than a 3-point decrease in fatigue score) across the 3 Time Points**




## Linear Regression Models

### Multiple Linear Regression Analysis

Multiple linear regressions (baseline, mid-point and end-point) were conducted to assess the impact of several possible independent variables on the dependent variable of fatigue. While sample size was determined for the primary aims of this study, the Green[135] formula for determining sample size needed was applied post hoc to determine the number of predictors we could use in the multiple linear regressions with sufficient power. Calculation showed that, for a small sample size of 26, only two independent variables should be used (*N* needed for a regression with 2 IVs: 8 + 1.5 = 9.5 = L; for a large effect of $R^2$ = .26; then $f$ = .26/(1-.26) = .26/.74 = .35; $N$= L/f = 9.5/.35 = 27). Among the potential variables, we did not include laboratory values as independent or as control variables since all laboratory results were in the normal range, and were only available from prior to the baseline visit (approximately 3 months before baseline visit). We excluded: (a) the concomitant use of neo-adjuvant hormonal therapy (ADT) because the results of the t-test showed that there were no significant differences between those on ADT and those not on ADT in fatigue across time (baseline vs. midpoint: t = -.72 (24), *p* = 0.48; baseline vs. end: t = -.41(24), *p* = 0.69; and, midpoint vs. end: t = -1.64, *p* = 0.11); and, (b) age, because even though the descriptive data showed some variability (*range* = 52-81, *SD* = 7.56), the correlation between age and the dependent variable baseline FACT-F was very weak (*r* = 0.06; age and FACT-F). In addition, if we consider as possible independent variables those obtained at the time the fatigue measures were taken, then the latter variables will be important for factoring in or for controlling their effect on measures of fatigue.[136] Weight was taken at each time point, but we excluded body mass index (BMI) because as with age, regardless of some variability (*M* = 29.35, *SD* = 4.16), it had no independent relationship with fatigue (*r* = 0.09; BMI and FACT-F). MET was taken at each time point, but we excluded MET as we did age and BMI, regardless of some variability (*M* = 2,415.78, *SD* = 3,888.54), because although the correlation between MET and the dependent variable at baseline FACT-F was moderate, it was not significant (*r* = 0 .31 *p* >.05, MET and FACT-F see Table 11). Among the variables that we selected to investigate for their contribution to fatigue were the depression measure (HDRS) and Sleep disturbance (PROMIS-SD). Results of the descriptive statistics and regression analysis will be presented next. All statistical testing used an alpha of 0.05.

Table 11

*Correlations among the Baseline Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F) and Age, Body Mass Index (BMI), Metabolic Equivalent of Task (MET), PROMIS-Sleep disturbance (PROMIS-SD), and the Hamilton Depression Rating Scale (HDRS) scores*

| **Variable** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| 1- Age | 1.00 | | | | | |
| 2- BMI | -.48* | 1.00 | | | | |
| 3- MET | -.29 | -.03 | 1.00 | | | |
| 4- PROMIS-sleep disturbance | -.15 | -.30 | -.13 | 1.00 | | |
| 5- HDRS | -.10 | -.24 | -.09 | .68** | 1.00 | |
| 6- Total FACT-F scores | .06 | .09 | .31 | -.70** | .69** | 1.00 |

* $p \leq .01$; ** $p \leq .001$

**Examination of Assumptions for Regression Analysis**

The analysis was conducted with data for 26 participants. Examination of the main assumptions for regression demonstrated that: (a) data were normally distributed as the Z-Residual Histogram showed an approximate normal curve, and the P-P Plot showed dots approaching to be on a line; (b) linearity was not an issue since the examination of residual plots showed a linear relationship; (c) constancy of variance represented no concern since the scatter plot of standardized residuals against standardized predicted values showed no clear pattern; (d) the obtained value of Durbin-Watson of 2.10 (between 1.5 to 2.5) suggested that the data points were independent; (e) outliers did not seem to be an issue because the standardized residual ranged between -1.79 to 1.90 (between -3.5 to + 3.5); (f) co-linearity, however, was an issue. As shown in Table 11, the bivariate correlation between baseline HDRS and baseline total PROMIS-SD score was moderately high ($r$ =.68; $p$ <.001). The skewness and kurtosis for each variable also was examined. The skewness ($S$) ranged from -0.84 to 2.48. Kurtosis ($K$) ranged from -2.05 to 7.99. The results of PROMIS-SD at baseline were ($S$ = .645; $K$ = -.337), at midpoint ($S$ = .395; $K$ = -1.067), and at end ($S$ = 1.139; $K$ = .424). At baseline, MET ($S$ = 2.342; $K$=5.445) and HDRS ($S$ = 2.075, $K$= 4.432), at midpoint, MET ($S$ = 2.244; $K$ =5.128), and at end, MET ($S$ = 2.562; $K$ = 6.578) and HDRS ($S$ = 2.524, $K$ = 6.803), with a skewness above one and kurtosis above three that suggests that these variables do not follow a standard normal distribution.

**Baseline Bi-variate Correlations**

The two variables considered for inclusion in the regression analysis was the PROMIS-Sleep disturbance and the HDRS. FACT-F scores were significantly associated with sleep disturbance scores ($r$ = -.70, $p$ = .001), indicating that persons who perceive feeling less fatigue tend to have less sleep disturbance. Similarly, the FACT-F was negatively correlated with HDRS scores ($r$ = -.68, $p$ = .001), indicating that persons who have less depression symptoms tend to perceive feeling less fatigue. However, because the PROMIS-SD scale was strongly correlated with HDRS ($r$ = .68, $p$ =.001), there was co-linearity between these two variables. Despite this co-linearity, we decided to include both in the regression analysis because each was so strongly related to fatigue.

When depression and sleep disturbance are enter separately, they both explain significant variability and when they are entered in a step-wise fashion each provides additional explanation. Therefore, multiple regression was conducted on the baseline data using the simultaneous method to enter the independent variables for the analysis. The linear combination of these variables was significantly related to FACT-F scores: $R^2$= .56, *adjusted* $R^2$=.53, $F_{(2, 23)}$ = 14.84, $p$ <.001. Thus, in the model the adjusted R-squared value revealed that 53% of the variance in total FACT-F scores was explained by the independent variables (PROMIS-SD and HDRS). The model summary of the multiple regression is presented in Table 12.

Table 12

*Summary of Multiple Regression Analysis for Independent Variables to Explain Baseline Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F)*

| **Variable** | ***b*** | ***SE*** | ***Beta*** | ***t*** | ***Sig.*** |
|---|---|---|---|---|---|
| Constant | 52.74 | 3.33 | | 2.75 | .012 |
| PROMIS-sleep | -.41 | .18 | -.43 | -2.03 | .03 |
| HADS | -1.58 | .78 | -.38 | -2.04 | .05 |

$R^2$ = .56; *Adjusted* $R^2$ = .53; SE of Estimate = 6.44 $F_{(2,23)}$ = 14.84 $p$ <.001

Examination of these results showed that baseline sleep disturbance variability emerged as a strong explanatory variable of fatigue (*b* = -.41, *SE* = .18, *β* = -.43, *t* = -2.03, *p* = .03). Similarly, the HDRS, reflecting the symptom of depression, also emerged as a significant explanatory variable of fatigue (*b* = -1.58, *SE* = .78, *β* =-.38, *t* = -2.04, *p* = .05). These results show that both variables are having a strong effect, despite their co-linearity, and both are statistically significant. Thus, higher total FACT-F scores (meaning less fatigue) was associated with lower depression (HDRS) and lower sleep disturbance (PROMIS-sleep disturbance).

**Midpoint Multiple Linear Regression Analysis**

The same analysis was conducted using Midpoint Fatigue as the dependent variable and midpoint measures of the independent variables, using the simultaneous enter method. Similar to the baseline bi-variate correlations, midpoint sleep disturbance (PROMIS-SD) and midpoint depression (HDRS) were strongly correlated with fatigue (FACT-F) and were correlated with each other (see Table 13).

Table 13

*Correlations among the Mid-point Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F) and PROMIS-Sleep disturbance (PROMIS-SD), and the Hamilton Depression Rating Scale (HDRS) scores*

| **Variable** | **1** | **2** | **3** |
|---|---|---|---|
| 1- PROMIS-sleep disturbance | 1.00 | | |
| 2- HADS | .71** | 1.00 | |
| 3- Total FACT-F scores | -.71** | -.63** | 1.00 |

Note: * p < .01; ** p < .001

This midpoint linear model also was statistical significant: $R^2$ = .54, *adjusted* $R^2$ at .50, ($F_{(2, 23)}$ = 13.34, $p$ < .001). However, only midpoint sleep disturbance emerged as a significant predictor in this analysis ($b$ = -.62, $SE$ = .23, $\beta$ =-.53, $t$ =-2.66, $p$ = .01). It is likely that depression did not become a significant predictor because sleep got credit for most of the shared variability between these co-linear variables Table 14 presents the results of this linear regression.

Table 14

*Summary of Multiple Regression Analysis for Independent Variables to Explain Mid-point Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F)*

| **Variable** | ***b*** | ***SE*** | ***Beta*** | ***T*** | ***Sig.*** |
|---|---|---|---|---|---|
| Constant | 55.54 | 3.78 | | 14.68 | .001 |
| PROMIS-SD | -.62 | .23 | -.53 | -2.66 | .010 |
| HDRS | -1.59 | 1.27 | -.25 | -1.25 | .22 |

$R^2$ = .54; *Adjusted* $R^2$ = .50; *SE of Estimate* = 6.34; $F$ $(_{2,23})$ = 13.34, $p$ < .001

**End of Treatment Multiple Linear Regression Analysis**

In another multiple regression, the dependent variable end-point FACT-F was regressed on end-point values of PROMIS-SD and HDRS as independent variables. Similar to the baseline and midpoint regressions, sleep disturbance (PROMIS-SD) ($r$ = - 0.75, $p$ =.001) and depression (HDRS) ($r$ = -.75, $p$ =.001) were strongly correlated with fatigue (FACT-F), and were correlated with each other ($r$ = 0.77, $p$ = .001) (see Table 15).

Table 15

*Correlations among the End-point Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F) and PROMIS-Sleep disturbance (PROMIS-SD), and the Hamilton Depression Rating Scale (HDRS) scores*

| **Variable** | **1** | **2** | **3** |
|---|---|---|---|
| 1- PROMIS-sleep disturbance | 1.00 | | |
| 2- HADS | .77** | 1.00 | |
| 3- Total FACT-F scores | -.75** | .75** | 1.00 |

Note: * p < .01; ** p < .001

The linear model also was a significant for end-point fatigue (FACT-F): the $R^2$ value was similar to the baseline model at .64, as was the *adjusted* $R^2$ at .61, ($F_{(2, 23)} = 20.45$, $p < .001$). Similar to the baseline model both end-sleep disturbance (b = -.43, SE = .19, β = -.43, t =-2.23, p = .04) and end depression ($b = -1.86$, $SE = .87$, $\beta = -.42$, $t = -2.13$, $p = .04$) remained significant explanatory variables in this analysis. Table 16 presents the results of this linear regression.

Table 16

*Summary of Multiple Regression Analysis for Independent Variables to Explain End-point Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F)*

| **Variable** | ***b*** | ***SE*** | ***Beta*** | ***t*** | ***Sig.*** |
|---|---|---|---|---|---|
| Constant | 62.19 | 2.92 | | 17.90 | .001 |
| PROMIS-SD | -.43 | .19 | -.43 | -2.23 | .04 |
| HDRS | -1.86 | .87 | -.42 | -2.13 | .04 |

$R^2 = .64$; *Adjusted* $R^2 = .61$; *SE of Estimate* = 5.39; $F_{(2,23)} = 20.45$, $p < .001$

In summary, fatigue (at baseline, midpoint and end-point) was regressed on sleep disturbance and depression at each time-point. At each time point the linear combination of these variables was significantly related to fatigue. In all three regressions, sleep disturbance emerged as the stronger explanatory variable of fatigue. While baseline and end-point depression reached statistical significance as an explanatory variable of fatigue, depression was not significant at midpoint likely due to the co-linearity between sleep disturbance and depression. Given the similarity of the correlations between these variables and fatigue, the role of sampling variability in these findings cannot be ruled out.

## Findings for Aim 2

*Aim 2: To assess gene expression changes from baseline to midpoint of EBRT among Hispanic PR men receiving EBRT for non-metastatic prostate cancer.*

Peripheral blood (2.5 mL) was collected from each subject at baseline and midpoint of EBRT to explore changes in gene expression using PAXgene blood ribonucleic acid (RNA) tubes (PreAnalytiX, Hombrechtikon, OH). Total RNA was extracted using the PAXgene™ Blood RNA system (Qiagen, Frederick, Maryland) according to the manufacturer's instructions described under the methods section. The quantity of total RNA was measured by a spectrophotometer at optical density of 260 nanometers. RNA quality was assessed using the RNA 6000 Nano LabChip® on a Bioanalyzer Agilent 2100 (Agilent Technologies, Palo Alto, CA). Total amount of good quality RNA was adequate to proceed with the experiment (see Table 17). A minimum of 50 ng of total RNA as starting material is suggested in order to proceed with the microarray experiment.

Table 17

*RNA quality data shows adequate amount of starting good quality total RNA material to proceed with the microarray experiment.*

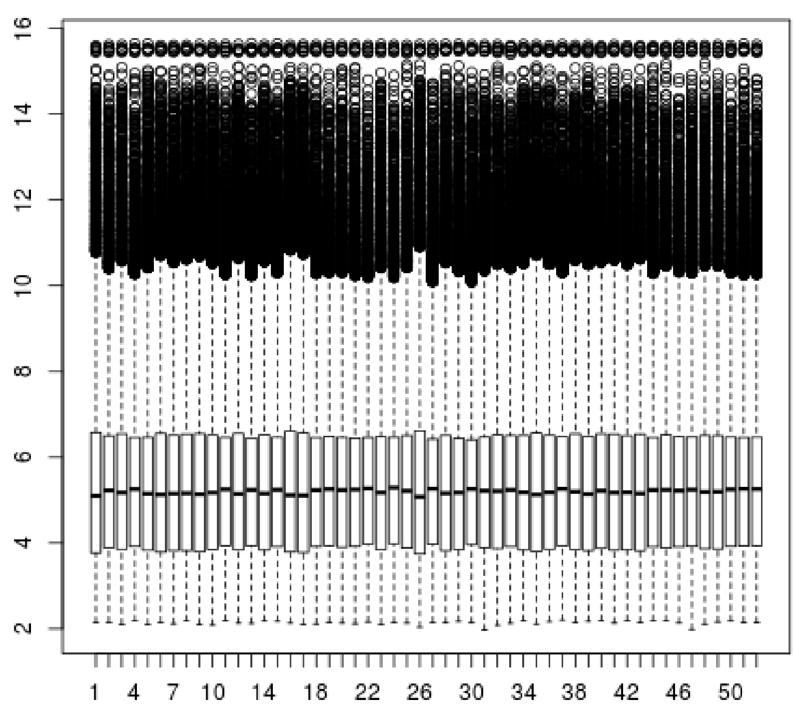
| ID | ng/ul |
|---|---|
| 143 | 86.93 |
| 127 | 52.63 |
| 142 | 77.04 |
| 138 | 97.74 |
| 151 | 102.13 |
| 125 | 105.38 |
| 118 | 164.43 |
| 141 | 197.05 |
| 116 | 87.43 |
| 101 | 61.47 |
| 117 | 96.7 |
| 115 | 120.62 |
| 112 | 94.68 |
| 144 | 126.06 |
| 152 | 69.36 |

RNA purification, cDNA and cRNA synthesis, amplification, hybridization, scanning and data analyses were conducted by one laboratory technician following standard protocols as described under the methods section. Affymetrix microarray chips (HG-U133 Plus 2.0, Santa Clara, California) were used for gene expression analysis. The Affymetrix HG-U133 Plus 2.0 microarray chip is comprised of more than 54,000 probe sets and 1,300,000 distinct oligonucleotide features, which analyzed the expression level of over 47,000 transcripts, including 38,500 well characterized human genes. Affymetrix GeneChip Command Console (AGCC, 3.0 V) was used to scan the images for data acquisition (see Figure 6, p. 67).

**Global Gene expression analysis**

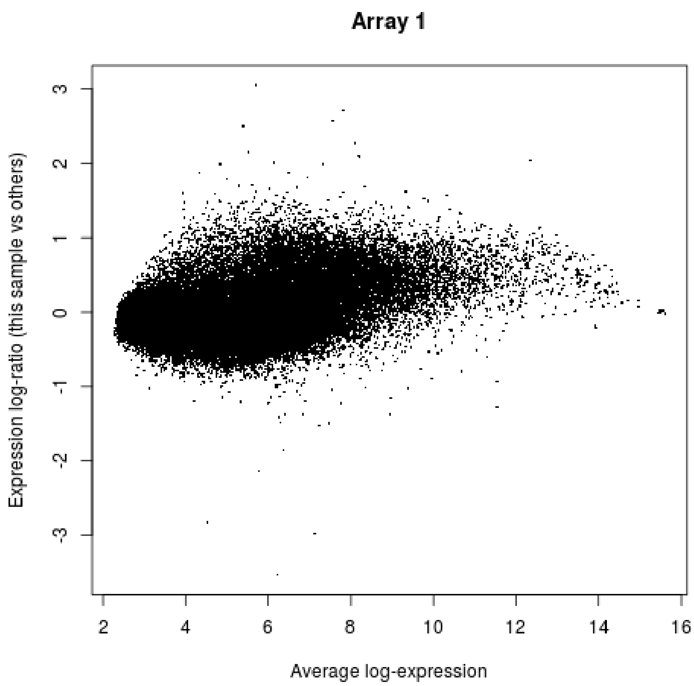
Affymetrix CEL files (the file format that stores the results of the intensity calculations of the pixel values of the scanned image from a microarray chip) were imported into *LIMMA*[132] package in R. Background correction, quantile normalization, and summarization expression calculation were conducted with the Robust Multichip Average (RMA) algorithm and the linear model with *LIMMA* package. In addition to the laboratory quality control steps, after RMA, data quality was assessed via per-subject box plots and MA plots. The subjects box plots shows that all arrays are within the expected range and that the distributions are similar to each other (see Figure 8).

**Figure 8. Boxplots of all Genes in each Array on the x-axis (n = 52 arrays), and the log base 2 of the Expression Values on the y axis**



In addition, the MA plot figure shows that no further normalization after RMA is needed because the distribution of the data of most of the genes (points on the y-axis are located at 0, since Log (1) is 0 (see Figure 9).

**Figure 9. MA Plot of Log-intensities Ratio where M (y-axis) Represents the Mean-difference between the Specified Array and the Artificial Average Array, and A (x-axis) is the Average Expression Value of that Gene in all Arrays.**




After the normalization step and the data quality check, gene expression differences between midpoint and baseline of EBRT were assessed using the linear modeling features available in the *LIMMA* package. This analysis consists of fitting a linear regression model for each gene to determine if genes were differentially expressed using a modified $t$-test that shrinks non-differentially expressed gene effect sizes towards zero (or fold-change towards 1). To adjust for the testing of thousands of genes, we used the approach of Benjamini-Hochberg to estimate the false discovery rate (FDR). To account for statistical significance, genes with a FDR < 0.01 were considered candidate differentially-expressed genes.

Three hundred seventy genes (130 up-regulated and 243 down-regulated) were differentially expressed from baseline to midpoint of EBRT FDR <0.01 (see Appendix R). Figure 10 illustrates a volcano plot of results for all genes for determining differentially expressed genes from baseline to midpoint of EBRT. This plot shows that a significant amount genes are differentially expressed, where a significant upward trend of ferredoxin reductase *FDXR* expression (Log Fold-Change = 0.496086; FDR = 4.0 x $10^{-6}$) was noted.

**Figure 10. Volcano Plot Showing -log10 (p-value) versus Fold Changes in the y- and x-axis respectively of Differentially Expressed from Baseline to Midpoint of EBRT**

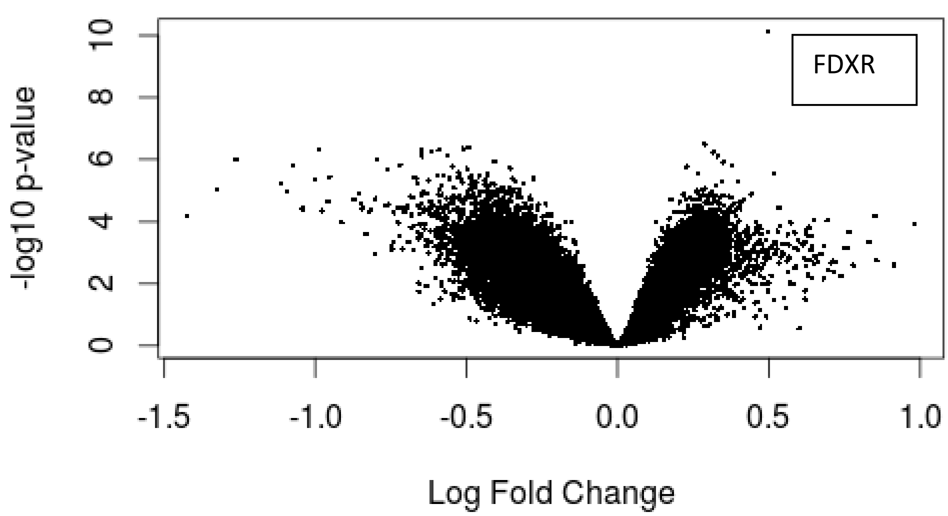



Table 18 presents the list of genes with FDR < 0.005. Five genes (*FDXR, YIPF6, AEN, SLC28A1, CSRP2*) were significantly up-regulated and 25 genes *(PRRC2B, NFATC1, CHMP7, RSBN, PPP1R3E, BPTF, LDLRAP1, MYCBP2, FOXP1, TCF7, ZBTB20, HSP90AB1, JADE2, NAPEPLD, MYC, ADARB1, ITPR1, CD79A, FAIM3, RAB30, ABLIM1, P2RX5, PAX5, BACH2, and FCRLA*) were significantly down-regulated at midpoint of EBRT compared to the baseline expression levels. It is plausible that more down-regulation occurred because the cells were over-stimulated by radiotherapy or by prostate cancer for this period of treatment, and the expressions of the receptor protein were decreased in order to protect the cell.[86]

Table 18

*The probes, gene symbols, names, Log(Fold-Change), regulation direction (Up/Down), p-values and FDR that were differentially expressed in the microarray experiment at α FDR <0.005.*

| **Probes** | **Symbol** | **Name** | ***Log(Fold-Change)*** | **Up/Down Regulation** | ***p-value*** | ***FDR*** |
|---|---|---|---|---|---|---|
| 207813_s_at | FDXR | ferredoxin reductase | 0.496086 | ↑ | 7.42E-11 | 4.06E-06 |
| 221234_s_at | BACH2 | BTB and CNC homology 1, basic leucine zipper transcription factor 2 | -0.98963 | ↓ | 4.86E-07 | 0.003632 |
| 221602_s_at | FAIM3 | Fas apoptotic inhibitory molecule 3 | -0.64948 | ↓ | 5.25E-07 | 0.003632 |
| 221601_s_at | FAIM3 | Fas apoptotic inhibitory molecule 3 | -0.76284 | ↓ | 2.2E-06 | 0.005014 |
| 223287_s_at | FOXP1 | forkhead box P1 | -0.49372 | ↓ | 4.04E-07 | 0.003632 |
| 224838_at | FOXP1 | forkhead box P1 | -0.5481 | ↓ | 1.7E-06 | 0.004576 |
| 224837_at | FOXP1 | forkhead box P1 | -0.52587 | ↓ | 2.7E-06 | 0.005447 |
| 240052_at | ITPR1 | inositol 1,4,5-trisphosphate receptor, type 1 | -0.618 | ↓ | 5.44E-07 | 0.003632 |
| 202431_s_at | MYC | v-myc avian myelocytomatosis viral oncogene homolog | -0.59249 | ↓ | 4.92E-07 | 0.003632 |
| 207560_at | SLC28A1 | solute carrier family 28 (concentrative nucleoside transporter), member 1 | 0.316246 | ↑ | 5.98E-07 | 0.003632 |
| 235308_at | ZBTB20 | zinc finger and BTB domain containing 20 | -0.51191 | ↓ | 4.54E-07 | 0.003632 |
| 219361_s_at | AEN | apoptosis enhancing nuclease | 0.330497 | ↑ | 7.99E-07 | 0.003642 |
| 1555779_a_at | CD79A | CD79a molecule, immunoglobulin-associated alpha | -0.648 | ↓ | 7.94E-07 | 0.003642 |
| 212660_at | JADE2 | jade family PHD finger 2 | -0.56362 | ↓ | 7.73E-07 | 0.003642 |
| 232279_at | JADE2 | jade family PHD finger 2 | -0.48261 | ↓ | 4.15E-06 | 0.005447 |

| Probes | Symbol | Name | *Log(Fold-Change)* | Up/Down Regulation | *p-value* | *FDR* |
|---|---|---|---|---|---|---|
| 212313_at | CHMP7 | charged multivesicular body protein 7 | -0.40711 | ↓ | 1.18E-06 | 0.004277 |
| 235401_s_at | FCRLA | Fc receptor-like A | -1.07415 | ↓ | 1.58E-06 | 0.004572 |
| 210448_s_at | P2RX5 | purinergic receptor P2X, ligand-gated ion channel,5 | -0.72014 | ↓ | 1.57E-06 | 0.004572 |
| 212340_at | YIPF6 | Yip1 domain family, member 6 | 0.378059 | ↑ | 1.59E-06 | 0.004572 |
| 207030_s_at | CSRP2 | cysteine and glycine-rich protein 2 | 0.227633 | ↑ | 1.76E-06 | 0.004576 |
| 205254_x_at | TCF7 | transcription factor 7 (T-cell specific, HMG-box) | -0.49858 | ↓ | 2.02E-06 | 0.004805 |
| 200965_s_at | ABLIM1 | actin binding LIM protein 1 | -0.6754 | ↓ | 3.74E-06 | 0.005447 |
| 203865_s_at | ADARB1 | adenosine deaminase, RNA-specific, B1 | -0.59477 | ↓ | 3.2E-06 | 0.005447 |
| 207186_s_at | BPTF | bromodomain PHD finger transcription factor | -0.45831 | ↓ | 3.5E-06 | 0.005447 |
| 200064_at | HSP90AB1 | heat shock protein 90kDa alpha (cytosolic), class B member 1 | -0.52668 | ↓ | 3.82E-06 | 0.005447 |
| 232279_at | JADE2 | jade family PHD finger 2 | -0.48261 | ↓ | 4.15E-06 | 0.005447 |
| 57082_at | LDLRAP1 | low density lipoprotein receptor adaptor protein 1 | -0.48592 | ↓ | 3.39E-06 | 0.005447 |
| 201959_s_at | MYCBP2 | MYC binding protein 2, E3 ubiquitin protein ligase | -0.4865 | ↓ | 3.8E-06 | 0.005447 |
| 238722_x_at | NAPEPLD | N-acyl phosphatidylethanolamine phospholipase D | -0.57602 | ↓ | 3.42E-06 | 0.005447 |
| 211105_s_at | NFATC1 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | -0.34769 | ↓ | 4.24E-06 | 0.005447 |
| 221969_at | PAX5 | paired box 5 | -0.95211 | ↓ | 3.84E-06 | 0.005447 |
| 229001_at | PPP1R3E | protein phosphatase 1, regulatory subunit 3E | -0.43205 | ↓ | 3.55E-06 | 0.005447 |
| 212068_s_at | PRRC2B | proline-rich coiled-coil 2B | -0.27695 | ↓ | 4.16E-06 | 0.005447 |
| 229072_at | RAB30 | RAB30, member RAS oncogene family | -0.66549 | ↓ | 4.23E-06 | 0.005447 |
| 222789_at | RSBN1 | round spermatid basic protein 1 | -0.43139 | ↓ | 2.93E-06 | 0.005447 |
| | | | | | | |

One of the 5 up-regulated genes ferredoxin reductase (*FDXR*) has a *p*-value = 7.42E-11 and a FDR of 4.0 x $10^{-6}$, two were greater than 3.6 x 10-3 (*SLC28A1, AEN*) and the last two *(CSRP2, YIPF6*) were greater than 4.5 x 10-3. *FDXR* was the most significantly up-regulated (logFC 0.50, p=0.00000406) gene. Eight of the 25 down-regulated genes (*FOXP1, ZBTB20, MYC, ITPR1, FAIM3, BACH2, JADE2, CD79A*) had an FDR = 3.6 x $10^{-3}$, four (*CHMP7, P2RX5, FCRLA, TCF7*) had an adjusted *p*-value that was less than 4.8 x 10-3, and, thirteen (*PRRC2B, NFATC1, RSBN1, PPP1R3E, BPTF, LDLRAP1, MYCBP2, HSP90AB1, NAPEPLD, ADARB1, RAB30, ABLIM1, PAX5*) had an adjusted *p*-value that was less than 5.4 x 10-3 log fold change. A list was generated of the top 20 significant up- or down-regulated genes accounting for both statistical and biological significance (see Table 19). Genes with a FDR < 0.01 and a fold change >0.4 in either direction were considered top candidate differentially-expressed genes. Table 19 also shows the genes from this top 20 up or down-regulated differentially expressed genes that were also observed to be the top 20 up or down-regulated genes in Caucasian men treated with localized radiation therapy for non-metastatic prostate cancer when microarray was conducted on RNA collected from these men at baseline and midpoint of EBRT.[17]

Table 19

*Top 20 Up-regulated and Down-regulated Differentially Expressed Genes between Mid-point and Baseline based on Adjusted p value and Log Fold Change*

| **Up-regulated Genes** | | | | **Down-regulated Genes** | | | |
|---|---|---|---|---|---|---|---|
| ***S** | Genes Symbol | Gene name | Expression value | ***S** | Genes Symbol | Gene name | Expression value |
| √ | *XK* | X-linked Kx blood group (McLeod syndrome) | 0.982<br>FC=1.97 | √ | *MS4A1* | membrane-spanning 4-do-mains, sub. fam A | -1.112<br>FC=0.46 |

| Up-regulated Genes | | | | | Down-regulated Genes | | |
|---|---|---|---|---|---|---|---|
| | *FGFR1OP2* | FGFR1 oncogene partner 2 | 0.851<br>FC=1.80 | √ | *FCRLA* | Fc receptor-like A | -1.074<br>FC=0.74 |
| | *KLF1* | Kruppel-like factor 1 (erythroid) | 0.692<br>FC=1.61 | √ | *POU2AF1* | POU class 2 associating factor 1 | -1.042<br>FC=0.48 |
| | *SESN3* | sestrin 3 | 0.649<br>FC=1.56 | | *BANK1* | B-cell scaffold protein with ankyrin repeats 1 | -1.039<br>FC=0.61 |
| | *ITLN1* | intelectin 1 (galactofuranose binding) | 0.533<br>FC=1.44 | √ | *IGHM* | immunoglobulin heavy constant mu | -0.999<br>FC=0.39 |
| | *FDXR* | ferredoxin reductase | 0.496<br>FC=1.41 | | *BACH2* | BTB and CNC homology 1, basic leucine zipper transcription factor 2 | -0.989<br>FC=0.5 |
| | *DPM2* | dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit | 0.457<br>FC=1.37 | | *TCL1A* | T-cell leukemia/lymphoma 1A | -0.977<br>FC=0.53 |
| | *DPCD* | deleted in primary ciliary dyskinesia homolog (mouse) | 0.441<br>FC=1.35 | | *LINC00926* | long intergenic non-protein coding RNA 926 | -0.957<br>FC=0.51 |
| √ | *RHD* | Rh blood group, D antigen | 0.408<br>FC=1.32 | √ | *PAX5* | paired box 5 | -0.952<br>FC=0.51 |
| | *ZER1* | zyg-11 related, cell cycle regulator | 0.406<br>FC=1.32 | √ | *CCR7* | chemokine (C-C motif) receptor 7 | -0.856<br>FC=0.55 |

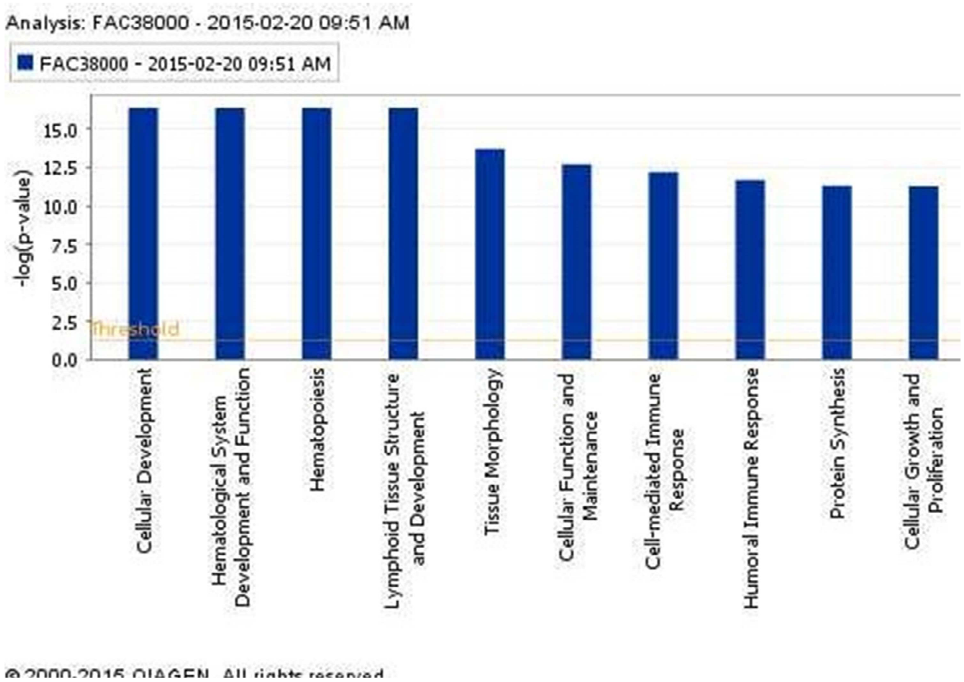
***S** √ = is a gene found in a previous study/publication (Caucasian study, Saligan et al., 2013) N=20

**Network and Pathway Analyses**

After the identification of differentially expressed genes from the microarray analysis, we employed network and pathway analyses to identify the key molecular and functional pathways associated with these differentially expressed genes. These functional networks suggested biological underpinnings of the possible physiological mechanisms that influence fatigue modulation during EBRT in this population.

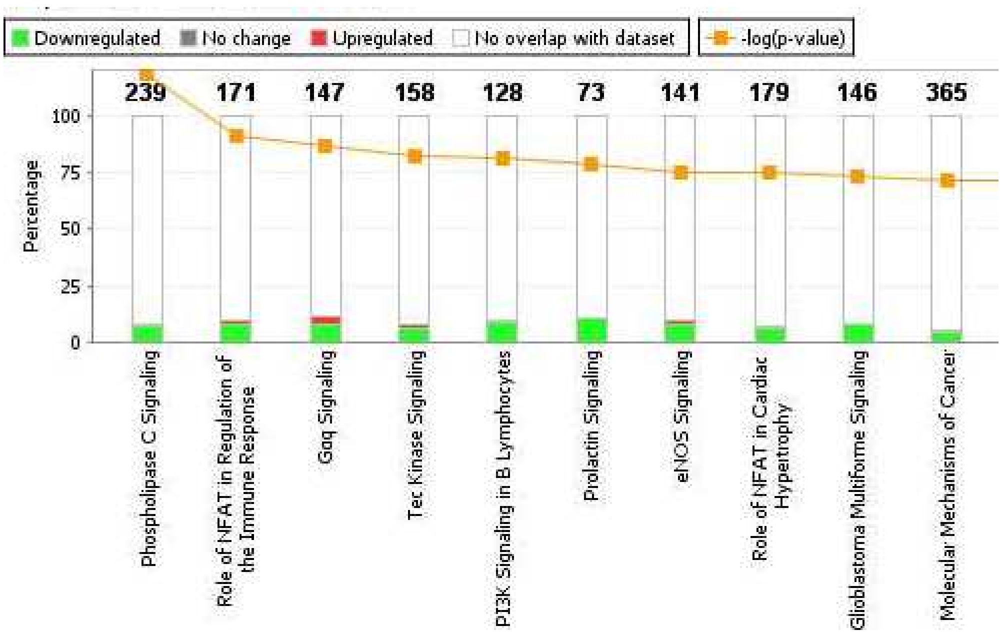
A significance level of $p < 0.0001$ was used for determining significant up- or down-regulated genes and for selection of genes for inclusion in the Ingenuity Pathway (IPA) (Ingenuity® Systems,www.ingenuity.com, Redwood City, CA) because of the exploratory nature of this study. The 646 probeset with a $p < 0.0001$ from midpoint to baseline of EBRT were subjected to pathway analysis using IPA. The cutoff log ratio was set at 0.4 providing the opportunity to analyze only those highly up-regulated and down-regulated differentially expressed genes ($N = 220$ genes). The IPA revealed several common biological networks that are associated with these 220 differentially expressed genes: (a) molecular and cellular functions such as cellular development, function and maintenance, growth and proliferation, protein synthesis, and, cell death and survival (see Figure11); (b) diseases and disorders such as cancer, hematological, immunological diseases, developmental disorders, and, organismal injury and abnormalities; and, (c) physiological system development and function such as hematological system development and function, hematopoiesis, lymphoid tissue structure and development, tissue morphology, and, cell-mediate immune response.

**Figure 11. Top 10 Molecular and Cellular and, Physiological System Development Functions of Radiotherapy-induced Gene Expression**



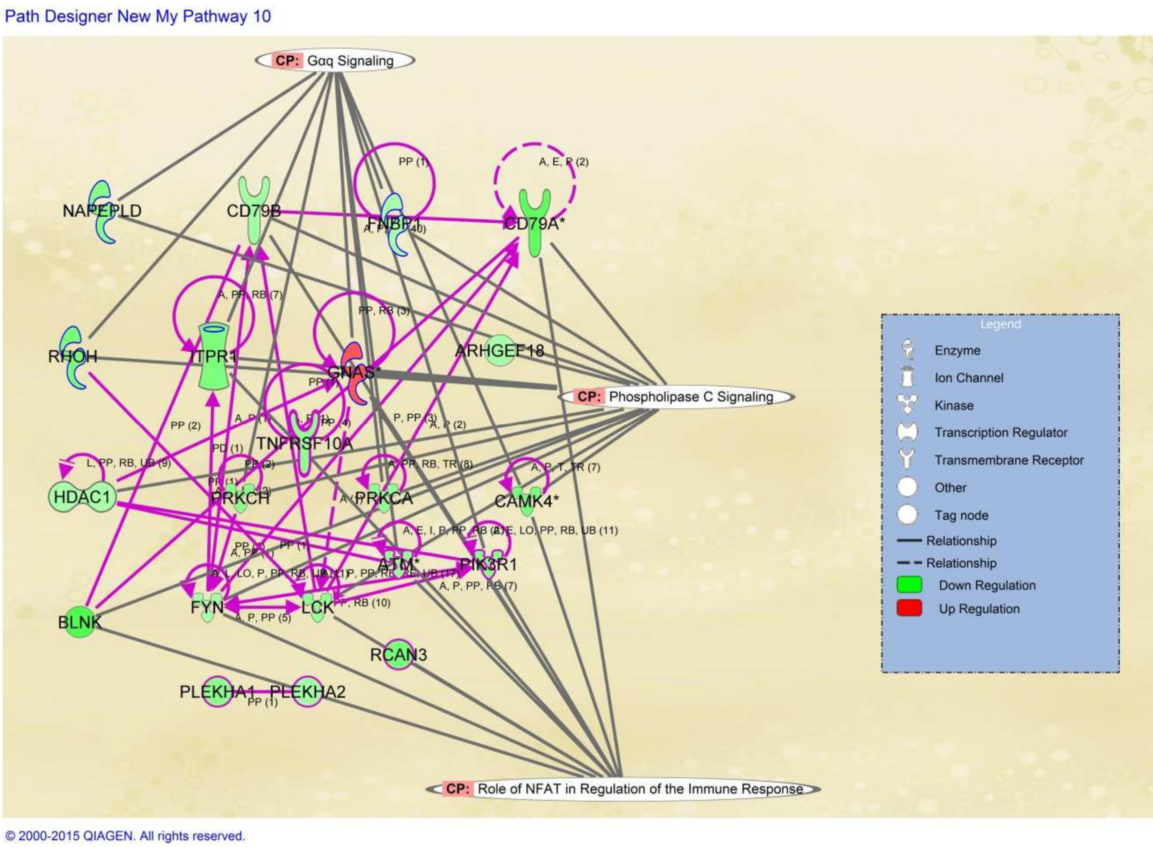

Further, Figure 12 shows the distinct canonical pathways of the differentially expressed probesets from the Ingenuity's Knowledge Base. Specifically, the significance of the association between the dataset and the identified pathway is represented: (a) as the percentage of regulated genes in our dataset divided by the total number of genes assigned to this pathway (this number is given above the pathway) and (b) by the *p* value calculated by Right-tailed Fisher's exact test (represented by their negative log-transformed value) determining the probability that each biological function and/or disease assigned to these networks was not due to chance alone. The top five canonical pathways that were associated with the 220 differentially expressed genes include: Phospholipase C Signaling, Role of NFAT in Regulation of the Immune Response, Gαq Signaling, Tec Kinase Signaling, and PI3K Signaling in B Lymphocytes (see Figure 12).

**Figure 12. Top 10 Canonical Pathways of Radiotherapy-induced Gene Expression**




A network of the top three canonical pathways (Phospholipase C Signaling, Role of NFAT in Regulation of the Immune Response, Gαq Signaling) and its associated differentially expressed genes was generated (see Figure 13). Coloring is based on the expression values of the genes, down-regulation in green and, up-regulation in red. The direct and indirect relationships from the Ingenuity knowledge database are shown by solid and dashed lines, respectively. The arrow indicates specific directionality of interactions.

**Figure 13: Top 3 Canonical Pathways of Radiotherapy-induced Gene Expression**




Findings for Aim 3

Aim 3: *To determine the association between changes in genes expression with changes in fatigue score from baseline to midpoint of EBRT in Hispanic PR men with non-metastatic PC.*

The list of the three hundred seventy-three genes (130 up-regulated and 243 down-regulated) that were differentially expressed from baseline to midpoint of EBRT (FDR <0.01; see Appendix R) was generated (Aim 2). However, the planned analysis of association between the changes in expression level of these genes with changes in fatigue scores from baseline to midpoint of EBRT was not conducted due to the unexpected finding that fatigue did not significantly change over the course of EBRT.

# CHAPTER V

# DISCUSSION

## Trajectory of Fatigue

The purpose of this exploratory study was to describe the trajectory of fatigue among Hispanic Puerto Rican men over the course of receiving EBRT for non-metastatic prostate cancer and to explore gene expression changes over the course of EBRT. We also were interested in comparing these findings with historical data of fatigue symptoms of Caucasian men with prostate cancer during EBRT. To our knowledge, this study is the first to examine the trajectory of fatigue before, at mid-point, and at the end of EBRT in Puerto Rican men with prostate cancer, an understudied population. We employed well-validated and reliable Spanish versions of instruments commonly used in other studies and that have been found sensitive to changes over time to assess fatigue and other symptoms. This sample was carefully assessed for potential confounding factors such as renal failure, uncorrected hypothyroidism, anemia, or chronic inflammatory disease resulting in participants with relatively homogeneous characteristics. In the gene expression analyses, measures were taken to correct for false positive results that may arise from high-through output techniques.

Fatigue can be a side effect in individuals undergoing radiotherapy that can negatively impact their health-related quality of life and physical functioning.[66,91] The FACT-F used in the present study is a widespread instrument used to assess fatigue among cancer patients. There are a number of publications that used the FACT-F with which we compared the scores obtained in this study, such as: normative scores for the U.S. general population ($N$ = 1010; 3.8% Hispanics)[137]; scores from prostate cancer patients receiving androgen deprivation therapy ($N$ =

23);[138] a national survey from cancer patients receiving chemotherapy and/or radiotherapy in the U.S. (*N* = 814; ethnicity not reported)[139]; and non- anemic cancer patients from the US (*N* = 113; 1.8% Hispanics)[137] with mixed cancer diagnoses under treatments.

At baseline and at end of treatment respectively, the Puerto Rican men of this sample had similar FACT-F mean scores (42.38, *SD* = 9.34 and 43.03, *SD* = 8.62, respectively) compared to those published using the same measure of fatigue for the U.S. general population (43.6, *SD* = 9.4).[137] At each of the three time point the Puerto Rican participants reported higher FACT-F mean scores, indicating less level of fatigue as compared to: Canadian prostate cancer men receiving androgen deprivation therapy (*M* = 37; *SD* = 24);[138] US non-anemic (*M* = 13.5; *SD* = 1.2)[137] cancer patients; and US cancer patients receiving chemotherapy and/or radiotherapy (*M* = 30.1, *SD* = 13.1)[139] using the same measure of fatigue.  Our smaller sample size may have contributed in part to the observed differences in fatigue, but it may also be due to the above studies dealing with more aggressive cancer types and/or more intense treatments.

Longitudinal studies have consistently reported that fatigue in prostate cancer patients increases over the course of EBRT.[16-17]  The results of this study do not support these findings. Unexpectedly, there were no significant differences in fatigue scores over time during EBRT (i.e., pre vs midpoint; pre vs end, midpoint vs end) in our Puerto Rican sample.  In comparison, the work by Saligan et al.[16-17] with a Caucasian sample found that, compared to baseline, fatigue increased significantly at midpoint and at completion of EBRT ($p$ = .001-.04) using the Piper Fatigue Score and the PROMIS-F.  Other longitudinal studies of Caucasian men with PC (Truong et al. *N* = 28;[66] Miaoskowski et al. *N* = 82[100]) found that compared to baseline, fatigue measured by the Brief Fatigue Inventory and the Lee Fatigue Scale increased significantly during EBRT.  While similar EBRT protocols and data collection time points were used, differences in assessment scales also may affect the observed results.[136] Although these instruments may have good psychometric properties, they may not lead to the same conclusions since they may also assess different aspects or characteristics of fatigue.[136] For example, the Piper Fatigue Scale assesses four dimensions of fatigue: behavioral/severity, sensory, cognitive/mood, and affective.[140] The FACT-F is unidimensional, like the Brief Fatigue Inventory, but the Brief Fatigue Inventory[141] assesses the interference of fatigue with activities of daily living and categorizes fatigue severity. Several studies have demonstrated that CRF is sufficiently unidimensional to be measured as such.[142] However, the work of Stein et al.[143] highlights that fatigue in cancer patients is a multidimensional experience composed of five dimensions: general fatigue, physical fatigue, emotional fatigue, mental fatigue, and vigor. With this concept in mind it is possible that some other dimension of fatigue not assessed in the present study might have changed over there course of EBRT. In addition to using different instruments to measure fatigue, these other studies had different sample sizes and were not limited to Hispanic participants. Due to these variations, direct comparisons between these studies and our study are limited.

Specific reasons why fatigue may have not worsened over the course of EBRT in our sample of patients are unclear. Recent evidence suggests that physical activity decreases during treatment for cancer. Interestingly, the participants of the present study showed an overall increase of physical activity (see Table 8) over the course of EBRT. Specifically, at baseline the level of total physical activity exceeded the 600 MET-minutes/week (required to fit in the "Moderate" category); at midpoint it was almost 3000 MET-minutes/week (required for the "High" category), and at completion it was even higher.

Although it appears that physical activity might have been related to fatigue scores in the present study, the Paired Samples t-test results were not statistically significant. The physiology of the effects of exercise on radiotherapy-related fatigue remains to be elucidated; the predominant physiological explanation appears to be related to inflammatory factors. It has been proposed that physiologically, radiation activates cellular signaling pathways that lead to the expression of pro-inflammatory cytokines that helps regulate the inflammatory process in response to tissue injury.[15] Regular aerobic exercise reduces inflammation also by triggering the release of pro-inflammatory cytokines from the skeletal muscle and inhibiting the production of inflammatory cytokines.[144]

There is increasing evidence focusing on fatigue and physical activity in prostate cancer patients undergoing radiotherapy which shows that exercise prevents fatigue through improvement in cardiovascular fitness, flexibility, and muscle strength. Monga et al.[145] conducted an interventional study of a supervised program of aerobic exercise three times a week for eight weeks on prostate cancer patients ($N = 11$) during radiotherapy. Participants of the exercise group showed improvements in post-radiotherapy METS and fatigue using the Piper Fatigue Scale, among other variables compared to pre-radiotherapy levels. In contrast, the control group showed significant worsening of post-radiotherapy scores on the Piper Fatigue Scale and no statistical change in MET. Windsor et al.[96] conducted a home-based moderate intensity exercise intervention for four weeks (walking for at least 30 minutes three times a week) using the Brief Fatigue Inventory in a larger sample size ($N = 33$) of prostate cancer patients during EBRT. Men in the control group reported significant worsening of fatigue scores from baseline to end of radiotherapy ($p = 0.013$) with no significant increases reported by participants in the exercise group ($p = 0.203$). Similar results have been obtained by other investigators with home-based interventions on prostate cancer patients [97,146] as well as among breast cancer patients.[147] This evidence suggests that physical activity appears to have potential benefits in reducing fatigue in prostate cancer patients during EBRT.[96,145] Taken together these findings suggest it is plausible that a good physical condition and maintenance of physical activity prior and during EBRT among the men of this sample may have contributed to no significant changes in fatigue. This finding also suggests the need for clinicians to be aware of the possibility of worsening of fatigue among those who present with lower physical activity, and consider promoting aerobic exercise in accordance with the guidelines of the American College of Sports Medicine on exercise for cancer survivors.[148]

Culture influences everyday social life as well as how people and their family and significant others respond to cancer diagnoses and treatment.[149] Family members have become aware that social support during cancer treatments might not only impact the course and success of treatment, but also may be beneficial in ameliorating the short and long-term side effects, such as fatigue.[150-151] It has been proposed that psychosocial support during cancer treatments helps patients in coping with the disease and is an important part of any comprehensive oncological care.[150] There have been few studies that have investigated if family/social support is a major positive factor to affect treatment-related symptoms.[150-151] Indeed, Brix and colleagues[150] found in patients with mixed cancer diagnoses undergoing RT treatment ($N = 239$), that those in need for psychosocial support reported significantly higher fatigue scores on the Multidimensional Fatigue Inventory than patients who were not identified as needing psychosocial support. In contrast, So et al.[151] found that fatigue measured by the Brief Fatigue Inventory was not significantly associated with social support measured by the Medical Outcomes Study Social Support Survey among Chinese woman undergoing treatment for breast cancer. The explanation provided by So et al.[151] about this finding was that, since the subjects were on active treatment, the effect of social support on pain and fatigue may have being relatively minimal compared to the effect caused by the toxicity of treatments.

In a qualitative study that explored cultural factors that influenced treatment decisions among Latino men, support received and role changes were among the emerging themes.[152] Under the theme of support, the investigator provided important information regarding Latino participants reporting receiving a lot of support from their immediate and extended family.[152] Specifically, a prostate cancer participant in the Carrion study[152] discussed how his friends and family members helped him accomplish tasks for which he was responsible in his home. Fatigue is a common side effect of RT frequently discussed during physician's conversations on treatment decisions in which the spouses of the Puerto Rican prostate cancer patients are most likely present. Fatigue is a multidimensional symptom comprising extreme tiredness and exhaustion that may result in poor physical, psychological, and social functioning.[150] Spouses of the participants of this study were identified as their primary caregivers and likely were the primary source of social support during treatment. Although not studies, as the PI witnessed family members accompanying patients to EBRT every day, it is possible that the participants received enough help from their family members to experience less 'causes' of fatigue. Within the cultural context, family members of the participants may have been so responsive to and supportive of the patients, that fatigue may not have been a major issue for them.

Spirituality also has been found as one of the most important coping mechanism that different cultures use to confront cancer diagnoses and treatment. When facing stressors, spirituality can be a great source of strength and comfort for some cancer patients and their families.[153] For example, it has been reported that prostate cancer patients with high levels of concern about their disease and prostate-related symptoms such as sexual and urinary dysfunction, were more likely to report increase in religiosity and spirituality after the cancer diagnosis.[153] There is evidence suggesting that spiritual-well-being is associated with the fatigue experienced by cancer patients during treatments. Indeed, Lewis et al.[154] study found that there was a significant inverse relationship between fatigue scores with spiritual well‐being and that spiritual well‐being also emerged as a significant negative predictor for fatigue using the FACT-F in 200 patients with mixed cancer diagnoses undergoing treatments. Similar results were reported by Kandasamy et al.[155] among 50 patients with advanced cancer in India. Whether patients who remained less fatigued also remained more spiritual during the trajectory of treatments is not known; no longitudinal study was found that could provide support for such a relationship.

Puerto Rican cancer patients have reported using spirituality for symptom management. For example, Gonzalez et al.[156] found that praying together and reading the Bible were common methods practiced by mothers of 65 Puerto Rican children/adolescents during cancer treatments when their cancer child was “afraid”, had difficulty sleeping or had mood changes. In addition, these PR mothers encouraged optimistic thoughts and communication among children and parents to alleviate the psycho-social and respiratory symptoms, and found it useful. Family-focused alleviation practices of seeking encouragement, advice, or help from relatives increased adherence to “Familismo” (familism)[157] values on the importance of building upon existing family strengths and coping strategies when promoting positive family functioning. Anecdotally, while at the recruitment center, the PI had the experience of listening to patients in the waiting room giving themselves positive reinforcement and talking about spiritual comfort. Despite what fatigue may represent to the men in this study, it is possible that any changes in fatigue the patients may have perceived was obscured by the feelings of comfort, strength, hope, and optimism that a spiritual state brings to individuals.

Situational factors also have been shown to affect the perception of fatigue. For example, Purcel et al.[90] found that being in a de facto relationship (compared with being married or single) was a strong correlate of fatigue among 210 cancer patients undergoing RT. Similarly, in a larger study Henry et al[139] found in a sample of 1,569 mixed-diagnosis patients receiving treatment that employed patients reported significantly higher fatigue on the FACT-F than unemployed patients. Henry et al.[139] also reported that those with at least a college degree reported lower fatigue than those with less than a college degree, and those with higher incomes reported significantly lower fatigue levels than those with lesser incomes. In comparison, in the present study the participants varied little on these situational characteristics since they were, for the most part married, well educated, and retired (probably receiving a fixed monthly income). The PI speculates that this finding suggests that issues related to being employed full-time that contribute to worsening of fatigue,[139] such as inability to take time off from work or to reduce working hours, and loss of income and productivity, may have not been present in our sample and also may account for the lack of increase in fatigue over the course of EBRT.

While fatigue did not worsen over the course of EBRT for this sample as a group, there was variability in fatigue across the sample. Also, some participants did experience worsening of fatigue while others experienced improvement in fatigue. When combined with the fact that most men in this sample did not change on their reported fatigue levels, it is not surprising that there were no group differences in fatigue over the course of EBRT. Our sample was too small to do any sub-group analyses on those for whom fatigue worsened. Further research with larger samples is needed to better characterize patients who experience increased fatigue, as well as those who experience improvement of fatigue.

Experimental studies documenting associations among genes related to oxidative phosphorylation, energy production, and mitochondrial membrane integrity with fatigue during RT have led to the proposition that alteration in ATP production may be a potential mechanism underlying RT-related fatigue.[16,27] Extrinsic factors such as EBRT can lead to oxidative stress.[27] Oxidative stress is the reflection of the inability of the mitochondria to detoxify ROS.[27,206] ROS damages the mitochondria, which in turn results in a reduced efficiency of oxidative phosphorylation, a reduction in production of adenosine-5'-triphosphate (ATP), and may lead to RT-related fatigue development.[206] Genomic changes could mediate some of the effects of ionizing radiation dysfunctional mitochondria.[27] The PI speculates that perhaps no changes in fatigue is a reflection of the combinations of antioxidant diet and supplements that can reduce ROS formation (less oxidative stress)[207] thus maintaining the patient’s ATP production and/or naturally restoring mitochondrial function. In particular, in the current study, mitochondrial-function relevant genes previously found to be associated with fatigue were not differentially expressed. Within this context, it is plausible that ATP production was maintained based on no mitochondrial gene expression resulting in no change in fatigue in the Puerto Rican sample.

*In vivo* and *ex vivo* studies suggest that dietary supplementation based on antioxidants may serve as a potential factor for improving behavioral symptoms such as stress and fatigue. For example, according to Pandareesh and Anand,[208] dietary L-carnitine (LC) plays a central role in fatty acid metabolism and in skeletal muscle bioenergetics through improving the energy charge by increasing the levels of ATP of skeletal muscle. Pandareesh and Anand’s [208] study showed that dietary L-carnitine and fat supplementation ameliorated induced physical fatigue in rats. Gramignano et al.'s [207] study suggests that fatigue, as measured by the Multidimensional Fatigue Symptom Inventory-Short Form, decreased significantly with L-carnitine (LC) supplementation administered orally at 6 grams per day for 4 weeks in cancer patients receiving chemotherapy. Carrillon et al.'s [209] trial of 12 weeks provided evidence that healthy individuals' (n=32) physical and mental fatigue as well as stress, were reduced with superoxide dismutase (SOD)-melon concentrate supplementation daily capsules compared to placebo (n=29). Stress was measured with the Cohen Perceived Stress scale and fatigue was measured with the Prevost Subjective Fatigue scale. The PI speculates that the consumption of an antioxidant rich diet (i.e. rich in vegetables and fruits) and/or supplements may have contributed to the attenuation of RT-related fatigue in the current study clinical population.

A sufficient dose and volume of radiotherapy can control tumor recurrence and the spread to nearby lymph nodes in locally advanced prostate cancer.[210] Overall, the total therapeutic radiation dose (ranging 61.2 Gy to 77.4 Gy) to tumor was relatively similar among patients in the current study. Despite the evidence that fatigue in prostate cancer patients increases over the course of EBRT,[16-19] longitudinal studies have also shown that patients symptoms peaked after treatment completion.[211] For example, Goineau e al.[211] found at 2 and 54 months post IMRT that patients reported significant and clinically relevant differences in fatigue with respect to baseline levels. Similarly, Wang et al.[212] found that the symptom burden of pain, fatigue, lack of appetite, disturbed sleep, and sore throat on patients undergoing chemo-radiation therapy for locally advanced non-small cell lung cancer peaked in severity after completion of therapy at week 8, and remained high for several more weeks, not returning to baseline severity until week 13 (about 5-6 weeks post-therapy). The current study collected fatigue data across time (at baseline, midpoint and at end of treatment) of EBRT and used their baseline fatigue assessment for comparison for the study; however, it is possible that the effect of accumulating radiation dose on the dynamic changes in patients' RT-related fatigue was not effective until after completion of RT. Specifically, the PI speculates that it is possible that a significant worsening of fatigue during EBRT did not peak at mid-point or end of treatment (week 8) but may have peaked later in response to accumulated radiation. Future research should examine the prevalence of post-radiotherapy-related fatigue and evaluate the need for more effective post-treatment symptom assessment and management.

Despite the recognition of conventional radiotherapy as a form of treatment for localized prostate cancer, over the last decade advances in radiation technology have led to the development of safer, high-dose radiotherapy techniques such as three-dimensional conformal radiation treatment (i.e. intensity modulated radiotherapy (IMRT).[210,213-216] Conventional radiotherapy had the limitation of requiring large safety margins due to the inability to identify the tumor precisely and did not allow for shaped blocking to shield normal tissues, thus potentially resulting in other organs adjacent to the prostate, such as bladder and rectum, receiving the same dose as the prostate tumor.[210,213-216] However, the development of the CT scan technologies not only allows a more precise definition of the geometry and location of the prostate and seminal vesicles, but also allows the CT-assisted EBRT planning.[210,213-216] Overall, three-dimensional conformal radiation treatment allows a more precise and safer delivery of high dose of radiation to the tumor target while reducing the chance of irradiating the normal tissue, improving treatment efficacy, and leading to less radiation-related side effects.[210,213-216] Specifically, the Puerto Rican men of this sample received IMRT. IMRT is a type of conformal radiation treatment that enables dose escalation by obtaining the information needed to individually prescribe a required dose distribution of the desired high-dose to the tumor target volume while treatment-related complications are potentially minimized.[216] Lips et al.[216] reported that patients in the IMRT group who received dose escalation to the prostate corpus showed significant improvements in QOL, less pain and urinary symptoms between baseline and one month after treatment compared to the group with no dose escalation (conformal radiotherapy with a dose of 70 Gy). Mangar et al.[213] reported that IMRT has resulted in reduced rectal toxicity when using doses greater than 80 Gy, can potentially escalate the dose to prostate tumor resistant cells, and/or can be used to extend the high-dose region to pelvic lymph nodes. Similar results of IMRT associated with the reduction of acute rectal symptoms, late rectal bleeding, and decreased urinary toxicity among non-metastatic prostate cancer patients have been reported by other investigators as well.[210,214] However, Lilleby et al.[215] found that fatigue, anxiety and QOL changed significantly over time in men with high-risk or locally advanced prostate cancer receiving both IMRT and conformal radiotherapy. Nonetheless, it is possible that the cells of surrounding normal tissues might have received less radiation due to the IMRT technique and improved position verification, resulting in less fatigue symptoms during RT in our sample. Longer follow-up is required to explore the possibility of late fatigue that might occur within 1 year of completion of radiotherapy.

**Predictors of Fatigue**

Consistent with our model "Gene Expression and Cancer-Related Fatigue " (see Figure 1, p. 5) our study found that sleep disturbance repeatedly emerged as a significant predictor of fatigue in the baseline, mid-point, and end of EBRT multiple regression analyses. These results suggest that participants who report more sleep disturbance are more likely to have more fatigue. This finding is consistent with that of others reported in the literature. Miaskowski et al.[100] found that the patients' baseline level of sleep disturbance measured by the General Sleep Disturbance

Scale predicted evening and morning fatigue in 82 men with prostate cancer during radiotherapy. Dhruva et al.[101] provided evidence that breast cancer patients with sleep disturbance prior to radiotherapy predicted the trajectory of morning fatigue but not the trajectory of evening fatigue. Similar findings regarding the relationship between sleep disturbance and fatigue also were reported by Stepanski et al.[158] in a large cohort of cancer patients undergoing treatment in which patients with sleep disturbance reported significantly more fatigue.

Depression also emerged as a predictor for fatigue at baseline and end-point of treatment regression models. This finding suggests that patients assessed with higher levels of depression are at greater risk for fatigue at the beginning of EBRT. Of note, none of the participants in the present study reached the cutoff score of 15 for clinically-concerning depression at any time point. One explanation for not showing as a predictor of midpoint fatigue may have been the co-linearity issue. Our measures of sleep and depression were strongly correlated in this study (baseline $r = .68$ $p < .001$, midpoint, $r = .71$ $p < .001$, end-point $r = .77$, $p < .001$). These findings also are conceptually congruent as sleep disturbance may lead to depression, or viceversa.[159] Other investigators have found sleep disturbance associated with depression.[158]

Another important limitation to be considered in interpreting these findings is that the selected measure of depression may not have been the best. The Hamilton Depression Rating Scale (HDRS) was originally designed for use with patients suffering from affective disorder of the depressive type (more severe) and not for use on normal subjects.[126] Consequently, the participants of this study were rated zero or one in the majority of the items of the scale that resulted in restricted variance. Further research may consider using other instruments for depression that are calibrated with normal populations such as the Patient-Reported Outcomes Measurement Information System (PROMIS).[160]

Nonetheless, these findings support the proposition of our model that an interrelationship exists between psychological, physiological, and situational factors and fatigue during EBRT. Redeker et al.[92] found that insomnia, fatigue, depression and anxiety were positively correlated with one another among 263 patients undergoing chemotherapy. Miaskowski et al.[100] also found that depression scores at baseline measured by the Center for Epidemiological Studies-Depression (CESD) Scale predicted variability in the trajectory of morning fatigue measured by the Lee Fatigue Scale in 82 men with prostate cancer during radiotherapy

It is difficult to visualize the occurrence of the single symptom of fatigue. An important consideration is to address the symptom cluster phenomena, in which sleep disturbance, depression and fatigue are common co-occurring symptoms experienced by cancer patients during treatment.[158] Indeed, Ho et al.[161] provided evidence for the manifestation of the symptom cluster of fatigue, sleep disturbance and depression among breast cancer patients, before, during, and after chemotherapy treatment. However, researchers are having difficulties understanding the causal direction among these symptoms.[158] For example, similar to other studies, in the present study increased sleep disturbance predicted increased fatigue. Other studies have shown that fatigued patients are at an increased risk to experience sleep disturbance.[162] A similar pattern has been identified in the relation between fatigue and depression, such that chronic fatigue is noted to cause depression,[162] and depressive patients are at increased risk to develop fatigue.[163] Sleep disturbance and depression have both been shown to commonly co-occur with fatigue.[161] This body of evidence provides support for the possibility of a bi-directional relation between these symptoms as integrated in our model. However, it is also true that both sleep disturbance and fatigue are more generally viewed as symptoms of depression.[163]

In the oncology arena, it is particularly important to understand this relationship among symptoms in order to develop and deliver targeted interventions in comprehensive oncology care. For example, given that our findings suggested that persons who sleep better and feel less depressed are less likely to experience fatigue, an intervention targeting depression and sleep disturbance would be expected to improve fatigue symptoms. In practice, it remains unclear which one of these symptoms comes first or why many cancer patients during EBRT experience these symptoms simultaneously. Additional exploration of these variables of interest is required to confirm if these are indeed the main predictors of fatigue, and by doing so, it will enable health care providers to better characterize fatigue in prostate cancer patients receiving EBRT. Further research using qualitative methodologies may be helpful to better understand why the Puerto Rican men in this study did not experienced changes in fatigue.

It also should be noted that none of the disease or treatment characteristics (i.e., pretreatment laboratory results; Gleason score, T-stage), were evaluated as predictors of fatigue at any time point because there was little variability in these variables: the laboratory results were all within normal range, and previous studies consistently reported that none of these measures were predictors of fatigue.[66,70] The exception was ADT hormonal therapy, but it was excluded because the results of the t-test showed that there were no significant differences between those on ADT and those not on ADT in fatigue across time, a finding supported in other studies.[66]

**Changes in Gene Expression**

EBRT remains a mainstay of prostate cancer therapy for Puerto Rican patients. Radiation therapy induces damage to the cell DNA that results in a cascade of events involving a network of signal transduction and transcriptional regulation.[164] This event stimulates a cellular stress response, including DNA damage recognition and cell cycle arrest, that leads to either DNA repair or apoptosis.[164] In our study, we used microarray technology to conduct a genome wide study focused on the identification of transcriptional changes resulting from the radiotherapy insult from baseline to midpoint of EBRT. The 20 most up- and down-regulated genes include p53-dependent genes, oxidative stress, and immune modulation related genes. To identify key functional categories and diseases within the differentially expressed genes we performed a review of literature using the Gene Ontology Consortium and Pubmed searches. In vivo and ex vivo studies have shown that differential expression of genes activating several physiological pathways may explain the mechanisms behind CRF.[15,165] These results suggest that the activation of these genes may have played an important role in the RT-related fatigue experience of this clinical population. These gene expression changes and the associated canonical pathways identified are in concordance with previous studies as described below.

During EBRT, there is active cellular apoptosis.[16] Consistent with this, the present study findings showed that apoptosis-related genes including ferredoxin reductase (*FDRX*) and sestrin 3 (*SESN3*) were up-regulated during EBRT. Ferredoxin reductase *FDXR* was the most up-regulated observed in this study. *FDXR* is regulated by the p53 family by DNA damaging agents in a p53-dependent manner, and by a mutated form of p53 that is involved in inducing apoptosis.[166] A previous study demonstrated that over-expression of the ferredoxin reductase protein increased the sensitivity of colorectal carcinoma cells to reactive oxygen species (ROS) 5-FU, and doxorubicin-induced cell death.[166] Specifically, the mechanism by which *FDXR* regulates ROS induced apoptosis is by hindering ROS from being detoxified by an antioxidant system.[166] ROS production is a critical process linked to RT-related fatigue.[16] It has been suggested that since ROS is mainly produced in the mitochondria, it may have an effect on oxidizing the mitochondrial pores resulting in disruption of the mitochondrial membrane potential that can consequently lead to cytochrome c release and apoptosis.[16,166]

Another study supported this hypothesis by reporting that ferredoxin reductase to be up-regulated following irradiation of lymphoblastoid cells at 3 Gy and 10 Gy doses, respectively, from 10 unrelated individuals.[164] In addition, FDRX also was observed to be up-regulated related to UV radiation damage stress response.[167] This observation was not only reported in radiation but also in chemotherapy where FDXR was one of the genes significantly induced by p53-mediated apoptosis after treatment with 5-FU through generation of oxidative stress in the mitochondria of colon cancer cells.[168] The current study findings and the supporting evidence strengthens our hypothesis that *FDXR* up-regulation accelerates ROS-induced apoptosis, which may contribute to the experience of fatigue in this clinical population.

The current study also revealed that the *SESN3* gene was significantly up-regulated during EBRT. Recent information establishes that all members of the Sestrin family are induced by oxidative stress linked to the metabolism of ROS and other reactive metabolites.[169] The gene SESN3 is known for being involved in the maintenance of physiological concentrations of intracellular ROS.[169] Intracellular ROS can influence cellular processes such as cell growth.[169-170] The accumulation of ROS in the mitochondria has been linked as an apoptotic stimulus[170] and to RT-related fatigue.[16] Nonetheless, SESN3 has been found to be activated by Forkhead box (FOXO) transcriptional factors.[169,170] In one study, it was observed that induction of SESN3 by Forkhead box O3 caused a transitory decline in ROS production and delayed Forkhead box O3-induced apoptosis of neuronal cell lines.[170] Similarly, Kopnin et al.[171] found that down-regulation of SESN3 in human Li-Fraumeni fibroblast cell line (MDAH041) cells caused increased ROS levels and chromosomal instability in cells expressing oncogenic RAS. Deregulation of intracellular ROS homeostasis has being found to play a role in the development of many diseases such as cancer, rheumatoid arthritis, and diabetes.[172] However, Sestrins can inhibit cancer cell growth through their role as suppressors of mTORC1 activity.[171] An earlier study demonstrated support for sestrin-2-related DNA damage upon irradiation and genotoxic drugs treatments on breast cancer cells through a poorly understood mechanism that may be related to the inhibition of mTORC1.[173]

Another gene of the Sestrin family, sestrin 1 (*SENS1*), have been found to be a potential fatigue-relevant gene. Broderick et al.[174] using the partial least squares methodology found that *SENS1* was the top influential gene from the microarray experiment that was significantly associated and influential to the symptom space composed of fatigue, depression, and sleep disturbance. Symptoms were measured with the Multidimensional Fatigue Inventory, Centers for Disease Control and Prevention Symptom Inventory, and the Medical Outcome Short Form-36 questionnaires. Specifically, *SENS1* was able to discriminate fatigued ($n$ = 75) from non-fatigued females ($n$ = 37) providing support for the oxidative stress involvement in chronic fatigue syndrome.[174] It is noteworthy that the up-regulation of *SESN3* observed in the present study could contribute to ROS accumulation contributing to the development of RT-related fatigue.

Kruppel-like factor 1 (*KLFLF1*), intelectin 1 (*ITLN1*), and dolichyl-phosphate mannosyltransferase polypeptide (*DPM*2) also were up-regulated in this study. KLF1 has been identified as a transcription factor involved in the regulation of erythroid differentiation.[175] Under homeostatic conditions, the expression of erythropoietin facilitates the production of erythrocytes.[176] However, under hypoxic conditions resulting from acute RT exposure, overexpression of erythropoietin maintains erythroid populations by the facilitation of proliferation and survival of erythroid progenitor cells. Voorhees et al.[165] proposed that since sustained glucocorticoid exposure stimulates proliferation of erythroid progenitor cells, and ligand-bound glucocorticoid receptor in conjunction with the transcription factor *KLFI* promotes erythroid differentiation, it is possible that sustained elevations in glucocorticoids levels in response to psychological stress contributed to erythropoiesis through erythroid progenitor proliferation. Expression of pro-erythroid transcription factor *KLF1* is restricted to erythroid cells and their precursors. Evidence from animal studies has demonstrated that mouse restraint stress has been useful in studying behavioral and biological symptoms associated with fatigue and depressive disorders.[165] Indeed, Voorhees et al.[165] found overexpression of *KLF1* at day 21 in mice exposed to chronic restraint stress. Sustained elevations in stress response also were evidenced by the elevation in stress markers, such as corticosterone levels, diminished body weight, diminished spleen and diminished thymic mass.[165]

The association between fatigue, psychological stress (e.g. stress, sleep disturbance, depression, and anxiety) and pain has been established.[158] Investigators have proposed that combining information from fatigue, psychological disturbance, and pain measurements, with gene expression data, will expand our ability to discover significant genes that might contribute to the understanding of the underlying etiology of this cluster of symptoms.[177] Thus, Voorhees et al.[165] results align with the present study finding that Kruppel-like factor 1 (*KLF1*) also was up-regulated. With this information in mind, it is possible that in the present study sustained elevations in glucocorticoid levels evoked by RT-related-fatigue and psychological stress might have occurred that consequently might have triggered a positive influence on erythropoiesis evidenced by over-expression of KLF1.

Of particular interest was the finding that *ITLN1* was overexpressed in the present study. Overexpression of the ITLN1 was observed on resected malignant pleural mesothelioma tissue and on adenoma of the colon.[178] That study implicated ITLN1, a human galactose-binding lectin, in cell differentiation, apoptosis, and recognition of tumor antigens, although the mechanism is not fully understood.[178] A link between *ITLN1* and fatigue resides in the potential role that adipokines (the secretory hormones released from adipose tissue) might play regulating multiple biological processes such as energy homeostasis and inflammation. Omentin is an adipokine that is codified by the genes ITLN1 and ITLN2.[177] RT-related fatigue it is a symptom triggered by inflammation and ATP dysregulation.[16-17] Omentin has been found to be differentially expressed in patients with nonspecific inflammation such as osteoarthritis[177] and obstructive sleep apnea.[179] Fatigue, similarly to obstructive sleep apnea, has been found to be associated with sleep disturbance and daytime sleepiness.[158] Although the underlying mechanism about this association remains to be elucidated, inflammation might be an etiological common factor. Indeed, Kurt et al.[179] found that plasma levels of omentin were found to be significantly higher in sleep apnea syndrome patients compared to the control group, although there was no significant correlation among sleepiness measured by the Epworth Sleepiness Scale and omentin levels in osteoarthritis patients. Kurt et al.[179] reported a small sample size as a possible limitation of the study.

Similarly, Gang-Li et al.[177]found no significant associations between serum omentin-1 concentrations and the Western Ontario McMaster University Osteoarthritis Index Scores in osteoarthritis patients. Gang-Li et al.[177] also acknowledged that the cross-sectional design and a relatively small sample size as a limitation. They concluded that further studies are needed to assess the anti-inflammatory effect of omentin-1 since there were no significant differences in serum omentin-1 concentrations between osteoarthritis patients and controls. Other investigators have reported that omentin had an anti-inflammatory role by preventing tumor necrosis factor-induced cyclooxygenase expression.[177,180] Thus, there is a great potential that overexpression of *ITLN1* in the present study represented an effort of the cells to release adipokines that mediate the inflammation process associated to RT-related fatigue.

The involvement of the immune system, and particularly T cells, in the fatigue experience of prostate cancer patients receiving EBRT has been proposed.[15] The list of the top 20 up-regulated and down-regulated differentially expressed genes of the present study (see Table 19 p.101 ) showed that, of the top 10 down-regulated differentially expressed genes, most were involved in immune system processes. The majority of these genes contains B cell-related annotations and/or is associated with B cell functions. Those involved in B cell-related annotations are: (a) *BACH2*, a B-cell-specific transcription repressor that has been shown to be a tumor suppressor in B-cell malignancy by enhancing apoptosis in response to oxidative stress;[181] (b) *BANK1*, an adaptor protein only expressed in B-cells that regulates calcium mobilization in response to B-cell receptor triggering and preventing hyperactive B-cell responses by attenuation of CD40-mediated;[182]; (c) *IGHM*, which encodes a B cell specific immunoglobulin, which is a trans-membrane receptor that has an important role in B cell development and signaling; (d) *TCL1A* (T-cell leukemia/lymphoma 1A), a proto-oncogene member of a multigene family that includes TCL1B and MTCP1, which is expressed in B cells. Those associated with B cell functions include: (a) *PAX5*, a B-cell-specific transcription factor that plays an essential role in B-cell development.[183] In the absence of PAX5, B-cells differentiate to other cell types such as T-lymphocyte or natural killer cells[183] reduction in PAX 5 expression has been linked to B-cells senesce (tumorigenesis) of a variety of cancers such as astrocytoma, B-cell-ALL, medulloblastoma, lymphomas and Wilm's tumors;[183] (b) *MS4A1*, the top down-regulated gene in both the present and the Caucasian studies; S4A1 is a member of the membrane-spanning 4A gene family, and encodes a B-cell surface molecule that functions in the differentiation of B-cells into plasma cells;[182] (c) *POU2AF1*, a B cell-specific transcriptional factor essential for B-cell maturation and germinal center formation that has been reported to contribute to susceptibility for Primary Biliary Cirrhosis among the Japanese Population[184] and for Multiple sclerosis among Europeans;[185] and (d) Fc receptor-like A encoded protein, selectively expressed in B cells, and which may be involved in their development.

The down-regulation of these genes, including the B cell specific immunoglobulin *IGHM* and regulators of B cell differentiation and activation, suggest a decrease in the B cell population among prostate cancer patients receiving EBRT. Commonly observed down-regulated genes from the current study and the previous Caucasian study[17] include *FCRLA, IGHM, PAX 5, BACH2, MS4A1, and POU2AF1*. Taken together, these results suggest that the expression levels of these B cell-related genes are affected by the disease and treatment status, regardless of ethnicity. Changes in differentially expressed genes related to B and T lymphocytes also were described among fatigued breast cancer patients.[84] These results might suggest that the observed changes in B cell related genes are specific to cancer-related fatigue. Evidence suggests that B-cell mediated inflammatory process might underline fatigue.[15,84]

The etiology of RT-related fatigue remains unclear. However, recent studies suggest that immune and inflammatory response trigger the fatigue experience. The involvement of the immune system, and particularly T cells, in RT-related fatigue has been reviewed by Saligan et al.[15] Expression analysis from the present study as well as from the Caucasian study showed that the most prominent group of differentially expressed genes were those involved in immune system processes. Such changes in gene expression, particularly altered functional B cell in fatigued patients and the role of the immune system, were described in detail by Myers,[186] and by Bradley, Ford and Bansal.[187] Myers [186] explains that, in the event of the body exposition to a pathogen, the antigens probably stimulate the response of the immune system. Specifically, macrophages (antigen-presenting cells) destroy the pathogen, evoking that antigens are moved to the cell surfaces of the macrophages to be recognized by circulating specific T cells (a type of white blood cells also known as leukocytes).[186] T -cells then bind to the macrophages and produce more T cells that recognize the particular antigens.[186] Among the different type of T cells, cytotoxic T cells play a role killing some types of antigens directly, while T-helper cells stimulate production of B cells (another type of white blood cell) that play an important role secreting an antibody that can destroy antigens.[186] Thus, the importance of B-cells resides in having multiple immune functions, such as antibody production, antigen presentation and regulation of the function and activity of other immune cells, (i.e., T-regulatory cells, NK cells and macrophages).[188]

These results suggest the involvement of B cells in the pathology of RT-related fatigue. Although the participants of the present study did not report experiencing bacterial infections that might have triggered a fatigue experience, it is possible that RT caused defects of B cell function that might underlie fatigue. Specifically, B cells play a role in producing antibodies and are potent antigen presenting cells.[187] Thus, impairment of B cell function or development can lead to recurrent infections, or a propensity to autoimmunity or allergy.[187] Other RT- related immune abnormalities also have been suggested.[15] For example, Landmark et al.[84]found that two of the gene sets that were expressed lower in fatigued breast cancer patients were involved in multiple myeloma and a diffuse large B-cell lymphoma. Landmark et al. [84]speculated that fatigue might engage some of the same pathways that are down-regulated in those type of B-cell disorders. Bradley et al.[187] further explains that B cell development in the bone marrow is an antigen independent tightly regulated process. After several rounds of B cell expansion take place, the functional light chains of the antibodies replace the surrogate light chains and pair up with the (mu) heavy chains, resulting in cell surface IgM expression eventually forming the B cell receptor (BCR).[187] BCR expression allows the negative selection of autoreactive B cells for elimination by apoptosis.[187] Deficiency of these processes may allow the later development of systemic autoimmunity.[187]

It is also possible that RT causes increased numbers of transitional B cells and naïve B cells that might overwhelm the B cell maturation process, which may consequently become suboptimal.[187] Alternatively, T cell help provided by cytokines may not support naïve B cells to develop into plasmablasts.[187] In addition, it is possible that RT caused one or more alterations in B cell maturation that may lead to an increased tendency to autoimmunity and a subtle humoral immune dysfunction, an active process by which the dysfunctional B cell maturation process contributes to symptomatology. Thus, if B-cell thresholds for cellular signaling, activation or proliferation are altered, this will increase the risk of self-reactive B-cells escaping and the potential of autoimmune disease.[187] Indeed, Bradley et al.[187] found that patients with moderate chronic fatigue had increased proportions of transitional and naïve B cells and reduced plasmablasts by which the dysfunctional B-cell maturation process might have contributed to the fatigue symptomatology.

In addition, Landmark's[84] study found that fatigued breast cancer survivors had an increased risk of B-cell lymphoma type cells expression that may indicate chronic immune activation or infection. Specifically, plasma cells develop from B cells in response to antigen presentation and T-cell activation. However, each plasma cell synthetizes and secretes one type of antibody that targets and binds to an antigen for destruction.[187] Thus, if RT causes changes in B-cell function, and if changes in B-cell function can be related to fatigue symptomatology somehow, then there may be a connection between RT and fatigue symptomatology.[15,17]

In the event of repression of B cell and T cells, if cancerous cells build up in the bone marrow, it might leave too little room for the production of red blood cells, white blood cells and platelets, which might make patients more susceptible to fatigue.[84] Landmak et al.[84] study on gene expression and fatigue among breast cancer survivors found that genes involved in multiple myeloma, B-cell lymphoma, B-cell recognition of a specific antigen and the B cells subsequent activation to mature B cells, also were down-regulated. In repression of genes related to B- and T-cell functions, RT can cause some defect of B cell memory or T cell dysfunction.[187] However, the total effects of B-cell depletion on the immune system are likely to be complex and time-dependent.[188] Cytokines that coordinate and stimulate the cellular process necessary for the production of antibodies are secreted by macrophages, T-helper cells, and B cells.[186] Dysregulation of B cells by RT can cause a differentiation of B cells into an increasing natural killer (NK) cell numbers and activation, reduced macrophage maturation and increasing tumor necrosis factor (TNF)—a secretion and decreased the suppressive function of T regulatory cells.[188] Our findings suggest that B cells might in fact be an additional tissue involved in RT-related fatigue that recently has been identified as multi-etiological, involving the dysregulation of several physiological and biochemical systems.[78]

One study showed that behavioral symptoms can be linked to altered gene expression of *IGH*M.[189] As in other studies,[158] the present study showed that RT-related fatigue was found to be associated with sleep disturbance. Earlier research has suggested that higher levels of fatigue during RT also were associated with pain.[158] Data from investigations with individuals with chronic abdominal pain have resulted in the recognition that sleep disturbance is the consequence of a complex cascade of biological events including inflammation.[189] Importantly, a number of genes pathways also have been implicated in other manifestations of side-effects of RT such as fatigue, depression and pain.[189] Studies examining changes in gene expression and changes in sleep disturbance suggest some common aspects to RT-related-fatigue etiology.[189] Based on expression analysis of data on 26 individuals with chronic abdominal pain, it was found that *IGHM* was one of the five genes that overlap between the pain and poor sleep quality group using the Pittsburgh Sleep Quality Index.[189] Similar to the present study, the majority of the differentially expressed genes comparing poor sleep quality to good sleep quality were down-regulated.[189] Consistency in microarray results suggests that down-regulation of *IGHM* may be related to the etiology of fatigue, poor sleep quality and pain. The hypothesis that changes in expression of genes related to B-cell function can echo some of the changes related to RT-related fatigue also might be supported by the results of the present study. Thus, further studies should investigate if prostate cancer men under EBRT with down-regulation of B cell-related genes are more prone to develop RT-related fatigue, and conversely, if men with a higher expression of B cell-related genes possibly might be carrying a protective effect against fatigue or if it is the disease process itself that decreases the expression of B cell-related genes in prostate cancer men.

*XK* and *RHD* genes are both transcription factors and are both observed to be up-regulated in this study and the previous study of Caucasian men.[17] The XK and the RHD genes belong to a family of genes called blood group (blood group antigens).[190] Recent evidence demonstrates that XK might play a role in transporting substances into and out of cells, maintaining cell structure, attaching to other cells and molecules, and participating in chemical reactions.[190] On red blood cells, the XK protein attaches to another blood group protein, the Kell protein.[190] Clinicians have reported the occurrence of decrease in hemoglobin during RT.[191] In the present study, differences in hemoglobin levels between baseline and midpoint of EBRT were not studied, nor differences in hemoglobin levels between fatigued and non-fatigued participants. Previous studies on prostate cancer patients during EBRT have found significant associations between fatigue and overexpression of genes related to hemoglobin synthesis.[16] Similar findings have been found by Landmark et al.[84] among fatigued breast cancer survivors. Since XK and the RHD genes are present within the cell membrane of red blood cells, overexpression of these genes offer support for a possible linkage between fatigue and the hemoglobin synthesis.

The mechanism involved in our Puerto Rican sample attenuation of RT-related fatigue fully may not be understood. However, a proposed hypothesis of RT-related fatigue etiology may involve ROS accumulation. As discussed above, several genes may play a role in this proposed mechanism. SESN3 is known to suppress oxidative damage.[169] Accumulation of ROS in the mitochondria serves as an apoptotic stimulus of the intrinsic death pathway.[170] Extrinsic factors such as EBRT can lead to oxidative stress.[27] Oxidative stress is the reflection of an imbalance of ROS and reactive nitrogen species (RNS) metabolism and inability of the cells (mitochondria) to detoxify ROS and RNS and other reactive metabolic intermediates.[169] While all members of the sestrin family are induced by oxidative stress by different induction mechanism,[169] *SESN3*, which is highly up-regulated in the present study, is stimulated by oxidative damage via activation of FOXO transcription factors.[170] FOXO3 also was up-regulated (*adjusted p value* <.02, log fold change 0.20) in this study. SESN3 knockdown caused an increase of FOXO3 inducing ROS and accelerating apoptosis.[170] SESN3, a FOX-inducible protein, has been shown to suppress oxidative stress-induced mTORCH 1 (target of rapamycin) activities, thus maintaining cellular energy during oxidative stress.[169-170] Although it is not clear that FOXO3 activated *SESN3* in our study, it is plausible that the antioxidant, AMPK-activating, and MTORC1 suppressing capabilities of the sestrin family contributed to the attenuation of RT-related fatigue in this clinical population.

Because the expression of 646 genes was found to be differentially expressed ($p$ <.00001) between midpoint and baseline of EBRT, we further explored the functional networks and canonical pathways in which these genes may be involved. The functional networks of the 646 differentially expressed genes in this study suggest that cellular processes (cellular development, function and maintenance, growth and proliferation, protein synthesis, and, cell death and survival) and especially immune response (including hematological system development) were the most active biologic pathway at midpoint of EBRT, which may be related to RT-induced cellular injury.

The top three identified functional pathways--phospholipase c signaling, role of NFAT in regulation of the immune response, and Gαq signaling--play an important role in intracellular and second messenger signaling, as well as in cellular immune response.[192] These pathways have been demonstrated in previous research to be involved in immune regulation and the complex signaling pathway that regulates cell dynamics.[192] However, since most of the genes from the top three pathways except *GNAS*, were down-regulated, this finding supports the idea that induction of RT in our sample may has been balanced by suppression of these genes based on the intensity of the damage.

As shown in Figure 13 p. 106, the canonical pathway is composed of 21 differentially expressed genes observed in this study. The canonical pathway is predominantly composed down-regulated genes; however, this is probably due to the fact that, although statistically significant, many of the up-regulated genes did not achieve the cutoff of 2-fold change (see Appendix R). Among the three top pathways, *GNAS, CAMK4*, and *ITPR1* were co-expressed in all three pathways. CAMK4 and ITPR1 are associated with calmodulin-dependent protein kinase activity, calcium-dependent protein serine/threonine kinase activity, intracellular signal transduction, ATP binding and inflammatory response.[193] Specifically, CAMK4 mediates calcium-dependent stimulation of dendritial growth, which is dependent on CAMK4-stimulated phosphorylation and activation of the transcription factor cAMP response element-binding protein (CREB).[193] CAMK4 is a multifunctional enzyme, which stimulates calcium-dependent, CREB mediated stimulation of dendritic growth and cell survival.[193] It has been reported that the CAMK4-CREB signaling cascade also inhibits apoptosis and promotes neuron and dendritic cell survival against various stresses. Bo Liu et al.[194] found a significant increase in apoptosis in cells repressing CAMK4 on mouse MIN6 B cells model. Therefore, the importance of repression of CAMK4 in the present study resides in the fact that apoptosis is the pathway necessary to make RT effective. Bo Liu et al.[194] provided evidence that overexpression of the constitutively active form of CAMK4 (DCaMK4) resulted in significant reductions in caspase-3/7 activities (whereas apoptosis was minimal) and stimulation of MIN6 B-cell division (promoted B-cell proliferation). Caspase-3/7 activity is universally increased during apoptosis.[194]

A possible link of *CAMK4* with fatigue further was investigated by Wu et al.[193] in transgenic mouse lines overexpressing constitutively active CAMK4 in skeletal muscles. Indeed, they found that skeletal muscles from these mice showed augmented mitochondrial DNA replication and mitochondrial biogenesis, up-regulation of mitochondrial enzymes involved in fatty acid metabolism and electron transport, and reduced susceptibility to fatigue during repetitive contractions.[193] Therefore, these previous reports on animals suggest that repression of *CAMK4* might be a common contributor to RT-related fatigue.

Dysregulated apoptosis caused by RT is a critical failure associated with RT-related fatigue. Bradford et at.[195] found that induction of apoptosis in G-292 human osteoblastic cells by exposure to etoposide or the inflammatory cytokine TNF-alpha promoted acute caspase-3/7 activity.[195] ITPR1, encodes the type 1 InsP3R.[195] InsP3Rs are intracellular calcium channels and key proapoptotic mediators.[195] The link of *IRTP1* in our study lies in the possibility that exposure to RT probably also repressed transcription of the *ITPR1* gene that encodes the intracellular calcium release channel implicated as a critical regulator of early apoptosis or programmed cell death.

*GNAS* was the only up-regulated gene common in the top three pathways. *GNAS* is likely to play a central role in regulating the biological process of signaling transduction. G proteins are known to trigger a complex network of signaling pathways such as the Gαq Signaling pathway.[192] In addition, *GNAS* have been linked to the regulation of activity of hormones by stimulating the activity of the enzyme adenylate cyclase.[192] Of the three genes of interest, more has been published on the relationship of GNAS and behavioral symptoms. *GNAS* was previously identified as a fatigue-associated gene among 112 female subjects with unexplained fatigue measured by the Multidimensional Fatigue Inventory.[196] Interestingly, *GNAS* have also been identified as differentially expressed in depressive patients with chronic fatigue syndrome measured by the Somatic and Psychological Health Report.[197] In summary, the relationship of *GNAS* with the development of behavioral symptoms relies on GNAS encoding the stimulatory

G-protein subunit alfa, which is involved in the generation of intracellular cAMP and plays a crucial role in energy expenditure and metabolism by mediating sympathetic.[198-199]

One of the most highly ranked pathways was Role of NFAT in Regulation of the Immune, which is predominantly marked by the up-regulation of *GNAS* and the down-regulation of BCR and the down-regulation of 8 genes (*IP3R, CSP, BCR, SLP65, LCK, FYN, CALM, P13K*). NFAT are a family of transcription factors expressed in several cell types of the immune system, therefore playing an important role in immune responses process.[192] NFAT are activated by stimulation of receptors coupled to Calcium-Calcineurin signals from various kinases.[192] The current study findings showed that the ATM serine/threonine kinase (log fold change= -0.510, p = .000043), calcium/calmodulin dependent protein kinase IVPIK3R1, PRKCA and PRKCH were significantly down-regulated, suggesting that the NFAT transcription factors are not activated during EBRT. Inflammation as a result of RT involves the activation and recruitment of phagocytes (macrophages, neutrophils), NK cells, complement system and secretion of cytokines like IL-1b, IL-6, TNF-a by activated cells that are essential for the host defense system[210]. None of these cytokines were differentially expressed in our study ($p > .05$). However, it also has been suggested that NFATc signaling pathway plays a role in dysregulation of inflammation.[200] The activation of the NFATc signaling pathway in macrophages leads to a hyperinflammatory effect with immune-pathologic consequences.[200] The acute inflammation during RT has been associated with fatigue.[15,17] Since NFATc isoforms actively and negatively are regulated in macrophages during acute inflammatory responses,[200] it is possible that any deregulation leading to NFATc activation (e.g. RT) might lead to excessive pro-inflammatory cytokine production and to the development to RT-related fatigue. NFATc activation in macrophages also has been found in pathologic disorders characterized by chronic TNF-α production, including rheumatoid arthritis.[200] In agreement with this supposition, fatigue has been found to be associated with TNF-mediated inflammatory state.

Another ranked pathway was the Gαq Signaling pathway. Similar to the previous pathways discussed above, *GNAS* was the only up-regulated gene (expression value 0.573); while the other genes associated with these pathways are down-regulated (*ATM, CAMK4, FNBPI, ITPR1, NAPEPLD, PIK3R1, PRKA, PRKCH, and RHOH*). After activation, the G-proteins route the signal molecules from cell surfaces receptors that are activated by ligands such as hormones, neurotransmitters and chemokines to regulate diverse physiological functions.[192] Whistler et al.[196] identified 839 fatigue-associated genes among 112 female subjects with unexplained fatigue. Unexplained fatigue was measured with the Multidimensional Fatigue Inventory. The authors did not provide the entire list of genes associated with fatigue; however, they provided the results of mapping fatigue-associated genes to pathways. These associated genes implicated six different signaling pathways including the G-protein signaling pathway. This similarity to the present study suggests that Gαq Signaling pathway might have played a role in fatigue development. Cell signaling pathways interacts with and regulates nearly all biological process associated with RT-related fatigue, such as cell growth, proliferation and apoptosis.[196] Apoptotic extrinsic pathway is triggered by activation of tumor necrosis factor receptor family, and the intrinsic pathway is triggered by various forms of stress such as radiation. Moreover, it has been postulated that the stimulatory G proteins modulate apoptosis induced by irradiation by regulating BCL-2 family expression in cancer cells treatment, and thus modulation of ROS-induced apoptosis.[201] A previous study also found dysregulation of BCL-2 expression to be associated with significant change in fatigue during EBRT in Caucasian men with non-metastatic prostate cancer.[21] These findings suggest that G proteins can protect various cells from apoptosis.[202] It is likely that during RT, the body uses apoptosis for eliminating unwanted cells and damaged cells to preserve homeostasis.[202] Thus, it is plausible that in our study at the midpoint of EBRT, the majority of our participants who did not experience RT-related fatigue may have better apoptosis-activating mechanisms to get reduce damaged cells from irradiation, compared to participants with significant change in fatigue.

The overall picture emerging from the findings of the present study supports the hypothesis that the etiology of CRF probably involves the dysregulation of several physiological and biochemical systems, such as oxidative stress, inflammation, immune modulation and hematopoietic dysfunction. Significant changes in expression of genes related to B-cell functions (*FCRLA, IGHM, PAX 5, BACH2, MS4A1, and POU2AF1),* inflammation *(ITLN1, CCR7*), hemoglobin synthesis (*XK and RHD*), and induced by oxidative stress (Sestrin family), in the same direction (up-/down-regulation) were demonstrated both in the present study with Puerto Rican patients and previously in the Caucasian study. Further studies will be needed to elucidate the possible roles of the B cell down-regulation and probably the oxidation phosphorylation process in these patients, i.e. ATP production in RT-related fatigue pathogenesis. Another approach will be to explore/confirm if the remaining differentially expressed genes from the top 20 (*FGFR1OP2, DPM2, DPCD, BANK1, BACH2, TCL1a, LINC00926, and ZER1*) might be related to fatigue development specifically among the Puerto Rican population. No study was located that reported a link between those genes and cancer-related symptoms or that reported that those genes are ethnicity specific. However, the concept of heterogeneity in people responses to RT is a possible explanation. Previous research among the Puerto Rican population has found heterogeneity-related differences in the field of pharmacogenetics.[203] Thus, ethno-geographic origin should be taken into consideration when conducting genomic studies.[203] For example, admixture is a type of gene flow among human population that may occur when individuals from two or more parental populations form a new hybrid population.[203] Most likely, the gene flow among Puerto Ricans islanders is a representation of the admixture among Amerindians, Spaniard, and West-African individuals as is gathered from history.[203] In support of this, Ruano et al.[203] study on 32,536 genotype assays from 332 SNPs in 196 cardiometabolic and neuroendocrine genes in 98 Puerto Rican islanders confirmed this trichotomous origin of Puerto Rican islanders with three approximately evenly divided clusters. In another study, Zuniga et al.[204] found that allelic frequencies of 15 autosomal short tandem repeat marker among 205 PR living in Massachusetts showed a 76.4 % genetic contribution of European, African 17%, and Amerindian genes 6.6%. Therefore, the PI speculates that the phenomenon of admixture may have played a role and may explain the possibility that the remaining differentially expressed genes from the top 20 (*FGFR1OP2, DPM2, DPCD, BANK1, BACH2, TCL1a, LINC00926, and ZER1*) are ethnicity specific.

Interestingly, in addition to similarities in gene expression observed in the present study and the Caucasian study, those genes that regulate the pathways and proteins thought to be underlying RT-related fatigue also are shared with studies of other psycho-neurological symptoms.[178-179,189] This evidence supports the hypothesis that common biological pathways (e.g. inflammation, immune dysfunction) might explain the cluster of symptoms cancer patients experience during and after treatment, such as fatigue, decreased activity, sleep disturbance, pain and stress. These symptoms are commonly named “sickness behavior.”[186] Thus, in response to infectious diseases, animals and humans might exhibit similar patterns, including lethargy, depression, anorexia, and reduction in grooming representing an adaptive response to illness.[186] It is believed that this response is driven to conserve energy and resources.[186] This pattern of sickness behavior also may demonstrate weakness, inability to concentrate, decreased interest in surroundings, decreased social and sexual interactions, anhedonia (inability to experience pleasure from normally pleasured life events), enhanced perception of pain and impaired learning.[186] In response to these characteristics, animals and individuals respond with an adaptive response to illness such as a pattern of increasing sleep, seeking warmth, and reducing energy devoted to seeking and grooming.[186] For example, Voorhees et al.'s[165] animal study reported that mice exposed to extended restraint stress protocols demonstrate similar biological symptoms as individuals experiencing psychological stress. These findings suggest that a new translational pathway [205] is needed specifically related to symptom-focused therapies. Also, the development of safe and effective treatments will be challenging. Challenges to be addressed may include the complex biochemical pathways underling the etiology of symptom cluster, and how to assess and manage the individual variability in symptom reporting in the trajectory of fatigue during and after cancer treatment.[205] However, the contribution of gene expression studies in developing personalized therapies seems promising. It will contribute to making use of individual differences in symptom experience to identify genetic and epigenetic risk factors for developing symptom trajectories.[205]

## Summary

In summary, the results obtained in the present study clearly illustrate significant differences in gene expression between mid-point and baseline of EBRT. While these differences in gene expression may offer a molecular explanation for the RT-related changes during EBRT, it suggests a biologic explanation behind the development of RT-related fatigue. Although RNA microarray results do not confirm definitive causation of change in genetic expression and behavior, the study findings are an important first step towards the goal of identifying a gene expression-based classifier able to discriminate fatigued versus non-fatigued Puerto Rican Hispanic prostate cancer patients using a simple blood test, could lead to improve treatment outcomes in this understudied population. Further, these study findings should stimulate nurses to better understand the mechanisms underlying fatigue intensification during cancer therapy. These data will provide nurses and other clinicians information to educate our patients about their symptoms so we can develop an optimal plan to manage their symptoms. Well informed oncology patients have better treatment outcomes, plan sound decisions for improved symptom management, and have enhance QOL following treatment.

Although it is unclear from our study whether the changes in gene expression we found in our study were specifically associated with fatigue, future research will be directed at providing direction toward the identified genes and pathways that may contribute to our understanding of the complexity of RT-related fatigue. This is particularly important for the Puerto Rican prostate cancer population because of the potential disabling symptom during and after EBRT.

## Limitations

Limitations of this study include the relatively small sample size (despite its appropriateness for our power analysis) and the unusual lack of change in fatigue over the course of EBRT that preventing addressing Aim 3. A larger sample would have permitted a subset analysis of those participants for whom fatigue worsened, something we were unable to do due to the small sample size. A limitation of this study may have been the use of a unidimensional measure of fatigue. As fatigue is multi-dimensional, (i.e. mental, physiological, and neuromuscular) it is possible our measure did not capture that part of fatigue that might have increased over the course of EBRT for more study participants. Another limitation of the current study was not having complete data of the complete blood cell counts as well as of urinary tract symptoms that might contribute to possible explanations of no changes of fatigue and/or no changes in sleep disturbance among the Puerto Rican prostate cancer patients. Findings from this study cannot be generalized to cancer patients with other diagnoses and under other types or combinations of treatments.

## Clinical implications

The use of EBRT in prostate cancer in Puerto Rico is a popular treatment option, and findings from this study suggest it may have a less detrimental effect on fatigue than in the Caucasian population. Physical activity has been shown to lessen fatigue severity among cancer patients during treatment. A good physical condition and maintenance of physical activity prior and during EBRT among the men of this sample may have contributed to no significant changes in fatigue. In the future, an exercise intervention may be reasonable approach for exploring how to reduce fatigue among those for whom fatigue worsens during EBRT. Also healthy diets consumed by participants may have increased the serum antioxidant levels, thus potentially reducing ROS formation leading to mitochondrial dysfunction that can lead to fatigue. Given the high association of sleep disturbance and depression with fatigue, health professionals should assess sleep disturbance and depression as well as fatigue and treat these as needed.

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,

# Appendix A

Recruitment Facility Support Letter

TOMÉ & UBIÑAS
Radio Oncology Center

April 7, 2014

Center for Scientific Review
National Institutes of Health
6701 Rockledge Drive, Room 1040 MSC 7710
Bethesda, MD 20892-7710

Dear F31 NRSA NINR Reviewers:

This letter is to confirm that Velda J. González will have permission to collect data for her study "Gene Expression and Fatigue in Puerto Rican men " at the Clínica Las Americas Tome &Ubiñas Radio Oncology Center anticipated to be from Fall 2014 to Spring 2016. Her data collection is contingent upon her successful completion of her dissertation proposal as well as obtaining an IRB approval at Kansas University Medical Center and at University of Puerto Rico Medical Sciences Campus.

In addition, this letter is also to confirm that Mrs. González will have a designated private interview/examination room and access to participants' medical chart to verify the inclusion exclusion criteria and to obtained selected heath information. As in a previous collaboration, I will introduce those participants interested in receiving more study information to the applicant who will be available at the facility. Further, I will provide her with support from my staff and I am committed to help her research succeed.

If you have any question, please do not hesitate to contact me at: (787) 764-5666.

Sincerely,

Jaime Tome, MD. DABR
Radiation Oncologist

Faculty: Jaime E. Tomé-Vilá M.D., D.A.B.R. W. Segundo Imbert M.D., D.A.B.R.

• PO Box 70321 • San Juan • Puerto Rico • 00936-8321 • Tel. 787-764-5666 • Fax 787-767-704
• Clínica Las Américas: Ave. Roosevelt 400 • Suite 109 • Hato Rey • Puerto Rico • 0091

**Appendix B**

Study Flyer

If you are a Prostate cancer survivor, over 40 years of age, who are about to start radiotherapy, you are eligible to participate in a study. If you are interested in more information, or would like to participate in the study, please call the co-investigator.

Velda J. Gonzalez

Co- Investigator

PhD. Nursing student/University of Kansas

University of Puerto Rico Cancer Center

(787) 457-8508

(787) 772-8300 x 11290

Velda.gonzalez@upr.edu

# Appendix C

University of Puerto Rico Institutional Review Board Approval Letter

**UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MÉDICAS**
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS

**OFICINA DEL RECTOR**
OFFICE OF THE CHANCELLOR

COMITE DE DERECHOS HUMANOS (IRB)
INSTITUTIONAL REVIEW BOARD

**Date:** April 24, 2014

**Protocol Number: 0160314**

**Principal Investigator:** Glorisa Canino

**Department / Division:** Academic Deanship

**Sponsor:**

**Title:** ***GENE EXPRESSION AND FATIGUE IN PUERTO RICAN MEN***

This is to certify that this research proposal/protocol was evaluated on April 24, 2014 and meets **full board** IRB review category. The research proposal was **approved.** The approval period for this study is **April 24, 2014** to **April 24, 2015**.

This action involves:
☒ New

The following documents were reviewed under this submission:
☒ Protocol
☒ Informed Consent Documents in English and Spanish Version
☒ Informative Sheet
☒ Survey Instrument
☒ Human Subject Certified
☒ Curriculum Vitae
☒ HIPAA Certified
☒ Authorization Letter
☒ HIPAA Identifiers

***Remember:***
- According to UPR Policies, if a proposed Project involves a component of research that falls under the jurisdiction of the Biosafety, Institutional Animal Care and Use and /or Radiation Safety Committees approval must be obtained from the appropriate Compliance Office.

For additional information please contact Human Research Subjects Protection Office at 787-758-2525 exts. 2510 to 2515; e-mail opphi.rcm@upr.edu.

Cordially,

Luz A. Muñiz, EdD
Chairperson IRB 3

bcg

1. Research must be conducted according to the proposal that was approved by the IRB.
2. Changes to the protocol or its related consent document must be approved by the IRB prior to implementation.
3. All serious or unexpected adverse events/drug reactions should be reported.
4. Each subject should receive a copy of the consent document, if appropriate.
5. Records must be retained for at least three years.
6. Any future correspondence should include the IRB identification number provided and the study title.

PO Box 365067, San Juan, Puerto Rico 00936-5067 Tel. / Phone (787) 758-2525, Exts. 2510 - 2515
**Patrono con Igualdad de Oportunidades en el Empleo M/M/V/I**
Equal Employment Opportunity Employer M/W/V/M

## Appendix D

University of Kansas Institutional Review Board Approval Letter

# The University of Kansas Medical Center

Human Research Protection Program

APPROVAL OF PROTOCOL

June 5, 2014

Lauren Aaronson
LAARONSO@kumc.edu

Dear Lauren Aaronson:

On 6/5/2014, the IRB reviewed the following submission:

| | |
|---|---|
| Type of Review: | Initial Study |
| Reviewing IRB: | KUMC |
| IRB#: | STUDY00001203 |
| Title: | Gene Expression and Fatigue in Puerto Rican Men |
| Investigator: | Lauren Aaronson |
| IRB ID: | STUDY00001203 |
| Expedited Category(ies): | (2)(b) Blood samples from others, (5) Data, documents, records, or specimens |
| Documents submitted for the above review: | • Recruitment Facility support letter<br>• Signed Scientific Merit Review Form<br>• Velda Gonzalez Expedite Project Description name<br>• UPR Mentor Dr. Canino biosketch<br>• UPR IRB approved informed consents Spanish/English<br>• Velda Gonzalez dissertation proposal<br>• UPR IRB letter of approval |

The IRB approved the study from 06/05/2014 to 6/4/2015 inclusive. Before 6/4/2015 or within 30 days of study closure, whichever is earlier, you are to submit a continuing review with required explanations. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR.

If continuing review approval is not granted before the expiration date of 6/4/2015, approval of this study expires on that date.

Your approved, stamped consent documents are found under the Documents tab, in your protocol. The consent forms posted in our electronic system are the only valid versions for documenting informed consent.

Mail-Stop 1032, 3901 Rainbow Blvd., Kansas City, KS 66160
Phone: (913) 588-1240 Fax: (913) 588-5771 humansubjects@kumc.edu

## Appendix E: Inclusion criteria check-List

Screening Sheet (to be filled by the co-Investigator)

**Are you…**

YES NO 1. ≥21 years of age

YES NO 1. Anon-metastatic PC patient scheduled to receive EBRT

YES NO 2. Concurrently receiving androgen deprivation therapy

**Have you ever had…**

YES NO 1. Major depression, bi-polar disorder, psychosis, within the past 5 years (e.g., Have you ever received a diagnosis from a psychiatrist, psychologist, or other mental health professional?

YES NO 1. Alcohol dependence/abuses, within the past 5 years

YES NO 2. Clinically significant fatigue (e.g. You may have been short of breath and the doctor may have told you that you had fluid in your lungs or that your heart was not pumping well.)

YES NO 2. Progressive or unstable disease of any body system (e.g. Heart failure, active hepatitis)

YES NO 3. Uncorrected hypothyroidism or anemia

YES NO 4. Second malignancies (e.g. has your cancer spread or metastasized to other parts of your body?)

YES NO 5. Concurrent chemotherapy with their EBRT?

YES NO 6. Chronic inflammatory disease (e.g. Osteoarthritis, rheumatoid arthritis)

YES NO 7. Are you on sedatives, steroids, or non-steroidal anti-inflammatory agents

YES NO 8. Lung problems such as asthma, emphysema, or chronic bronchitis

YES NO 10. Kidney problems

a. **If yes**, do you require hemodialysis or peritoneal dialysis?

# Appendices E & G

Informed Consent English



**UNIVERSITY OR PUERTO RICO**
**MEDICAL SCIENCE CAMPUS**
**INFORMED CONSENT FOR PARTICIPATION IN A RESEARCH STUDY**

**TITLE OF THE PROJECT:** GENE EXPRESSION AND FATIGUE IN PUERTO RICAN MEN

**Principal Investigator:** Glorisa Canino, PhD
**Co-Investigator:** Velda J. Gonzalez, RN, MSN

**Institutions:** Several Radiotherapy Centers

**TELEPHONE NUMBER 24-HOURS: (787) 457-8508**

**INTRODUCTION**

You have been invited to participate in a research study. This consent form explains the purpose of this study and what will we ask of you if you decide to participate. In this form we also describe the possible risks related to your participation in this study. Your participation is voluntary. Your decision to whether participates or not in this research study will not affect the quality or availability of the medical care you will receive.

Before you decide to participate in this study, you must read this consent carefully. This consent form may have words that you do not understand. Please, ask the principal investigator or her staff to explain any word or information that might not be clear to you. You will receive a copy of this document (so you can think about its content or discuss it with your family or friends) before making a decision of whether or not to participate in this study.

We are asking you to participate in this research study because you have been diagnosed with prostate cancer and you are scheduled to receive radiotherapy treatment.

**DESCRIPTION OF THE STUDY**

**WHAT IS THE PURPOSE OF THIS STUDY?**
The purpose of this research project is to learn if there is a relationship between Genes in blood and Fatigue Symptoms during Radiation Therapy for Non-Metastatic Prostate Cancer patients.

**WHO MAY PARTICIPATE IN THIS STUDY?**
This study is open for participation to men over 40 years of age that have been newly diagnosed with non-metastatic prostate cancer scheduled to receive Radiation Therapy. You will not be able to participate if you have: (a) cancer recurrence; (b) history of previous chemotherapy or

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From 04/24/14 TO 04/24/15

planned chemotherapy or braquitherapy with your Radiation Therapy; (c) are taking sedatives, steroids, or non-steroidal anti-inflammatory medications; (d) uncorrected hypothyroidism or anemia; (e) and or non treated mental illness or second malignancies.

**HOW MANY PEOPLE WILL PARTICIPATE IN THIS STUDY?**
For the whole study, we expect to recruit approximately 26 participants. The participants will be patients from several Radiotherapy Center of the Island.

**WHAT IS THIS STUDY ABOUT?**
Your participation will consist of 3 visits to your Radiotherapy Center. Each visit should take no longer than 45 minutes of your time.

Visit 1 prior to initiation of Radiotherapy.
Visit 2 days 19-21 after Radiotherapy.
Visit 3 days 38-42 after Radiotherapy.

During this visits you will be ask to answer several questionnaires related to possible side-effects of Radiotherapy and how you perceive your quality of life.

The questionnaires are the following:

- FACT-F (13 statements about fatigue)
- IPAQ-SF (9 items that asks about a seven-day recall of the amount of minutes spent in activity of four intensity levels specific types of activity)
- PROMIS-sleep disturbance (8 statements of how sleep quality has interfered with your daily activities)
- Demographic Form (10 items about your marital status, religious preference, and education level).

In addition, the investigator will request from you information about your health history (eg. weight, height) and if you have had experience any sign of depression such as feelings of guilt "Self-reproach, feels he has let people down."

Your medical record will be searched to obtain information such as the stage or size of the tumor, disease progression, and laboratory results. It is necessary to obtain this information from your medical record given that this information is difficult to remember by some patients.

Furthermore, all participants should consent to donate blood to examine which genes in blood may be related to Fatigue Symptoms during Radiation Therapy. We will draw 2 blood tubes (approximated 2 tablespoons of blood).

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From 04/24/14 TO 04/24/15

**ADDITIONAL INFORMATION**

The blood samples collected will be processed and frozen in a locked freezer until shipment to the National Institutes of Health in Bethesda, Maryland. Nor your name or any other personal information will be linked to your samples. Blood samples will be kept for approximately five years at Dr. Leorey N. Saligan, PhD, RN, Laboratory at the National Institute of Nursing Research, Bethesda, Maryland. In addition there is a possibility that the samples will not be completely used, therefore our collaborators may use it for future research. In this case, do you agree that your samples be used for future investigations?

___ I Agree______ Initials ___ I Do Not Agree______ Initials

**WHICH ARE THE POSSIBLE RISKS AND DISCOMFORT THAT YOU MIGHT EXPERIENCE BY PARTICIPATING IN THIS STUDY?**

Among possible risks and discomfort you might experience:

Discomfort or tiredness from answering the questionnaires. In the rare event a participant experiences severe distress, they will be referred for evaluation, counseling and follow-up services with the principal investigator Dr. Canino (clinical psychologist). Dr. Canino can provide follow-up services or may refer you to a mental health professional if needed.

Participants may experience pain, bleeding and/or hematomas as a result of blood drawing procedures. You might also experience dizziness, redness, irritation and in rare occasions infection.

The investigators will take all the necessary measures to minimize those risks including: resting time between questionnaires to prevent fatigue, and the blood samples will be taken by certified personnel.

**WHICH ARE THE POSSIBLE BENEFITS IF YOU DECIDED TO PARTICIPATE IN THIS STUDY?**
In this study you will not receive any medical treatment. It is probable that you will not receive any benefit from your participation in this study. In the future, other patients might benefit from the results of this study.

**WHAT ARE THE COSTS OF THE STUDY?**
You will not have to cover any additional cost if you decide to participate in this research study.

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From 04/24/14 TO 04/24/15

**WILL YOU BE PAID FOR YOUR PARTICIPATION IN THIS STUDY?**
You will not be paid for your participation in this study.

**WHAT WILL HAPPEN IF YOU DECIDE NOT TO PARTICIPATE IN THIS STUDY?**
You can decide not to participate in this study. If you do not participate, you will not answer the questionnaires and we will not take any other sample from you. Your decision to whether or not participate in this research study will have no effect in the quality or availability of the medical care that you will receive.

**WHAT INFORMATION WILL BE OBTAINED?**
If you decide to participate in this study, the investigator of the study will obtain your personal information. This may include your age, marital status, religious preference, education level, and occupation. In addition, you may feel upset and/or distressed with the possible sensitive or personal nature of the information that you will be asked to provide such as if you have experienced any sign or symptom of depression in the last 2 weeks.

The information from this research study will be used only for the purposes described in this informed consent. Information that could identify the patient will not be disclosed beyond the requirements for this research study. By signing this form, you will allow the research team that includes staff working on this study from the University of Puerto Rico Medical Science Campus and other entities described in the confidentiality section of this consent, to obtain, use and disclose the information described in this form. The information collected in this study could be shared with other researchers in the future, but only so that the information cannot identify you.

There is no expiration date for the use of this information.

It is possible that during the course of the study the participants may not have access to their research charts. However, you have the right to see and reproduce the record related to this investigation after the study is finished.

**AUTHORIZATION FOR THE USE AND DISCLOSURE OF HEALTH PROTECTED INFORMATION**

If you decide to participate in this study, the investigator of the study will obtain your personal information. This may include information that might identify you such as your name, telephone number and medical chart number. Your telephone number may be necessary for reminder of follow-up visits. Also, study investigators may obtain information like your answers to the questionnaires. This information might be shared with other team investigators and with the

Office for the Protection of Human Subjects in Research of the Medical Science Campus (UPR MSC IRB). The UPR MSC IRB could evaluate your medical record to obtain information or to monitor that the procedures of this study are being followed in a safe manner and according to the procedures indicated above.

The UPR Cancer Center will take all the necessary measures to maintain your personal information private. Any information obtained in connection with this study that can identify you will remain confidential as required by law. Federal regulations provide guarantees of privacy, security and unauthorized access to your information. It is our priority to protect personal information that can identify you, for this the following measures will be taken:

All information collected will be kept in locked files. Each participant in this study will be assigned a study number to avoid using other numbers that may identify you, such as social security number or driver's license. Survey results will be analyzed by using this number only and will not identify any individual in publications related to the study

You may cancel your authorization at any time sending a written note to the any of the investigators to the following address:

**Dra. Glorisa Canino, PhD**
Instituto de Investigación de
Ciencias de la Conducta
Universidad de Puerto Rico
Recinto de Ciencias Médicas
PO Box 365067
San Juan, PR, 00936
(787) 754-8624
E-mail: glorisa.canino@upr.edu

**Velda J. González, RN**
Centro de Cáncer
Universidad de Puerto Rico
PMB-371 Po Box 70344
San Juan, PR 00936-8344
(787) 772-8300 ext. 1129
E-mail:
velda.gonzalez@upr.edu

If you revoke this authorization, the study investigator will not be able to use your health-related personal information, unless some of the information is required to maintain the scientific integrity of the study. Information shared prior to the cancelation of this authorization could still be used by the investigators.

Authorization to use and share your health information is completely voluntary. Nevertheless, if you don't sign this document, you will not be able to participate in this study.

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From 04 24 14 TO 04 24 15

**WHAT CONPENSATION CAN YOU RECEIVE IN CASE OF HARM CAUSED AS A RESULT OF YOUR PARTICIPATION IN THIS STUDY?**

Given that all the procedures included in this study are part of the clinically-indicated procedures for diagnosis or treatment of your medical condition, any compensation for damages will be related to the medical management of your condition. Therefore, any case of physical or mental damages as a result of the procedures performed will be covered under the appropriate sources related to your medical care. The Medical Sciences Campus of the UPR will not offer any form of direct compensation for your participation in this study. However, by signing this document you are not waiving any legal right. You will also not receive any compensation as a result of any patent or discovery resulting from your participation in this study.

**WHO SHOULD YOU CALL IN CASE OF QUESTIONS ABOUT THIS STUDY?**

If you have any questions about this study or about your participation in this study, you may call the any of the investigators Dr. Glorisa Canino at (787) 754-8624 or Velda González at (787) 772-8300 ext. 1129 or 787-457-8508 after working hours. If there are no personnel available immediately, we will return your call as soon as possible.

If you have any question regarding your rights as a participant in this study you can call the Institutional Review Board of the UPR Medical Sciences Campus (787) 758-2525 ext. 2510 or 2515 or to their email address: opphi.rcm@upr.edu.

Do not sign this consent unless you have had the opportunity of asking questions and had received satisfactory answers to all of them.

If you decide to participate in this study, we will give you a copy of the signed consent with the approval seals of the UPR Medical Sciences Campus.

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From 04/24/14 TO 04/24/15

**INFORMED CONSENT**

I have read (or had been read) the above information related to GENE EXPRESSION AND FATIGUE IN PUERTO RICAN MEN and the content had been explained. I had the opportunity to ask questions. All my questions were answered. Freely, I consent to participate in this study. I authorize the use and share of my personal health information to the mentioned agencies in the section of authorizations in this consent for the purposes already described. The sign of this consent do not imply waiving of my legal rights.

__________________________
Name of participant

__________________________ ________________
Signature of Participant Date

__________________________
Name of the Investigator

____________________________________________________
Signature of the Investigator or Date
Designated person that obtain the consent

IRB APPROVED (MSC/UPR)
From 04/24/14 TO 04/24/15

# Informed Consent Spanish



**UNIVERSIDAD DE PUERTO RICO**
**RECINTO DE CIENCIAS MÉDICAS**
**CONSENTIMIENTO INFORMADO PARA LA PARTICIPACION EN UNA INVESTIGACION**

**TITULO DEL PROYECTO:** Expresión de genes y síntoma de fatiga (cansancio) en hombres Puertorriqueños

**Investigador Principal:** Glorisa Canino, PhD
**Co Investigadores:** Velda González, RN, MSN

**Instituciones:** Varios Centros de Radioterapia

**NÚMERO DE TELÉFONO LAS 24-HORAS:** (787) 457-8508

## INTRODUCCION

Se le ha pedido que participe en este estudio de investigación. Este formulario de consentimiento explica por qué se está realizando este estudio de investigación y que se le pedirá que haga si decide participar. En este formulario también se describen los posibles riesgos relacionados con su participación en este estudio. Su participación es voluntaria. Su decisión de participar o no en este estudio de investigación no tendrá consecuencia alguna en la calidad o disponibilidad del cuidado médico que reciba.

Este consentimiento informado podría contener palabras que usted no entienda. Por favor, solicite al investigador o a su personal que le explique cualquier palabra o información que no esté clara. Antes de tomar su decisión usted puede llevarse a su casa una copia no firmada de este consentimiento informado para pensarlo discutirlo con familiares o amigos.

Se le ha pedido que participe en este estudio de investigación porque usted tiene un diagnóstico de cáncer de próstata y va a recibir Radioterapia.

## DESCRIPCION DEL ESTUDIO

### ¿CUAL ES EL PROPOSITO DEL ESTUDIO?

El propósito de este estudio de investigación es el de aprender acerca de cómo los genes en la sangre pueden estar asociados con el síntoma de fatiga (cansancio) durante el tratamiento de radioterapia para el cáncer de próstata.

**¿QUIEN PUEDE FORMAR PARTE DE ESTE ESTUDIO?**
Pueden participar en este estudio hombres de 40 años o más que han sido recién diagnosticados con cáncer de próstata y van a recibir tratamiento con radioterapia. o podrán participar aquellos pacientes que: (a) el cáncer haya regresado; (b) tienen tratamiento previo o planificado con quimioterapia o braquiterapia; (c) utilizan regularmente medicamentos sedantes o anti-inflamatorios, (d) tengan enfermedad de tiroides o anemia sin trata, (e) y/o que tengan enfermedad mental sin tratar u otro diagnóstico de cáncer.

**¿CUANTAS PERSONAS PARTICIPARAN EN ESTE ESTUDIO?**
Esperamos reclutar un total de 26 participantes. Los participantes serán de distintos Centro de Radioterapia a través de la isla.

**¿CUANTO TIEMPO DURARA MI PARTICIPACION Y EN QUÉ CONSISTE EL ESTUDIO?**
Su participación en este estudio consistirá de 3 visitas a su Centro de Radioterapia. Cada visita durará aproximadamente 45 minutos.

Visita 1 Se realizará antes de comenzar radioterapia.

Visita 2 Se realizará de 19 a 21dias luego de comenzar radioterapia.

Visita 3 Se realizará de 38 a 42 días luego de comenzar radioterapia.

Durante estas visitas a su Centro de Radioterapia, se le pedirá que llene una serie de cuestionarios que nos ayudarán a entender los síntomas que sienta durante el tratamiento de radioterapia y como usted percibe su calidad de vida.

Los cuestionarios son los siguientes:

- FACIT-F (13 preguntas sobre fatiga)
- IPAQ-SF (9 preguntas sobre actividad física en los pasados 7 días)
- PROMIS-alteraciones relacionadas con el sueño – (8 preguntas de como su calidad de sueño ha afectado las actividades que realiza durante el día)
- Hoja demográfica - 10 preguntas que incluyen su estatus marital, preferencia religiosa y último grado académico completado

Además el investigador le hará preguntas sobre su historial de salud (ejemplo. peso, estatura) y si ha percibido en los últimos 7 días alguna señal o signo relacionado a depresión como por ejemplo sentimientos de culpa (ejemplo: "Si ha sentido que ha decepcionado a otras personas.")

De su expediente médico se recopilará información como tamaño y tipo de tumor, progresión de enfermedad, y resultados de laboratorio, ya que es información que le podría ser difícil de recordar.

Además, en este estudio le solicitaremos una muestra de sangre para evaluar que genes en la sangre se relacionan con la fatiga (cansancio) que usted pueda sentir durante el tratamiento de radioterapia. Se le tomarán dos tubos de sangre, lo que representa unas dos cucharadas de sangre.

**Información Adicional**

La sangre será guardada para luego enviarse al los Institutos Nacionales de Salud (NIH) en Bethesda, Maryland. Ni su nombre y ni la información de salud serán relacionados con la sangre que se envíen. Las muestras se mantendrán aproximadamente por cinco años en el laboratorio del Dr. Leorey N. Saligan, PhD, CRNP, RN, Instituto Nacional de Investigación en Enfermería, Institutos Nacionales de Salud (NIH) en Bethesda, Maryland. Debido a que la cantidad de sangre que se tomará es limitada; en la mayoría de los casos no sobra muestra para ser utilizada en otros estudios. Si sobrara muestra, ¿aceptaría usted que se use para estudios futuros que aún no se sabe cuáles serán?

______ Si acepto ______iniciales ______ No acepto _____ iniciales

**¿CUÁLES SON LOS POSIBLES RIESGOS Y MOLESTIAS DE PARTICIPAR EN ESTE ESTUDIO?**

Dentro de los riesgos e incomodidades relacionadas a este estudio se encuentran los siguientes:

- Posibilidad de sentir alguna incomodidad o cansancio al finalizar de contestar los cuestionarios· La co-investigadora podrá referir a la investigadora principal, la Dra. Canino (psicóloga clínica), aquellos participantes que se encuentren en crisis emocional durante o al finalizar de contestar los cuestionarios de cada visita. La Dra. Canino podrá ofrecer seguimiento o le referirá a un profesional de salud mental de ser necesario

Al tomar la muestra de sangre usted puede experimentar un dolor mínimo debido a la aguja. Además se puede formar un moretón o infectar el área de donde se tomó la muestra.

Los investigadores harán todo lo posible por minimizar los riesgos e incomodidades antes mencionados asegurándose de lo siguiente:

- Se le ofrecerá el tiempo de descanso que necesite para evitar fatiga.
- El proceso de toma de muestras de sangre será realizado por personal certificado que tomará las medidas adecuadas de limpieza a fines de minimizar el riesgo de infección.

**¿CUÁLES SON LOS POSIBLES BENEFICIOS DE LA PARTICIPACIÓN EN ESTE ESTUDIO?**
En este estudio usted no recibirá ningún tratamiento médico. Es probable que usted no reciba ningún beneficio personal por participar en este estudio. Con este estudio esperamos recopilar información que pueda ayudar a otras personas en el futuro.

**¿CUALES SON LOS COSTOS DEL ESTUDIO?**
Usted no tendrá que incurrir en gastos para participar en este estudio de investigación.

**¿SE LE PAGARÁ POR TOMAR PARTE EN EL ESTUDIO?**
No se le pagará por su participación en este estudio

**¿CUÁL ES LA DIFERENCIA ENTRE PARTICIPAR O NO EN ESTE ESTUDIO?**
Si no participa en este estudio, no se afectará su tratamiento médico. De no participar, usted no contestará los cuestionarios ni dará la muestra de sangre.

**¿QUE INFORMACION SE RECOPILARA EN ESTE ESTUDIO?**
La información que se recopilará en este estudio incluye la información que usted provea sobre sus datos personales como su edad, estatus marital, preferencia religiosa, grado académico más alto alcanzado y tipo de empleo. Además, en los cuestionarios se recogerá información sensitiva como por ejemplo si ha experimentado en las últimas 2 semanas algún síntoma de depresión.

La información recopilada en este estudio se usará solamente para los propósitos descritos en este consentimiento. La información que pueda identificar al paciente no se divulgará más allá de lo requerido para este estudio de investigación. Al firmar esta hoja, le permite al equipo de investigación que incluye al personal que trabaja en este estudio del Recinto de Ciencias Medicas de la Universidad de Puerto Rico y otras entidades descritas en la sección de "Confidencialidad" de esta hoja de consentimiento, obtener, usar y divulgar la información descrita en este formulario. La información recopilada en este estudio podría ser compartida con otros investigadores en el futuro, pero solo de manera que la información no pueda identificarlo.

La información recopilada en este estudio se mantendrá por un tiempo indefinido.

IRB APPROVED (MSC/UPR)
From 04/24/14 TO 04/24/15

Es posible que durante el curso de este estudio, lo participantes no tengan acceso a sus expedientes de investigación. Si lo desea, puede pedir esta información luego de que el estudio haya terminado.

**¿SE MANTENDRÁ PRIVADA SU INFORMACIÓN?**
Si usted decide participar en este estudio, el investigador del estudio obtendrá información personal sobre usted. La única información que lo podría identificar es su nombre, su número de teléfono y su número de expediente médico. Su número de teléfono se le solicitará en caso de ser necesario ofrecerle seguimiento por haber desarrollado alguna crisis emocional mientras contesta los cuestionarios. El investigador del estudio podría obtener información además, como sus respuestas en los cuestionarios. El investigador del estudio podría dar información sobre usted a la Oficina Para la Protección de Participantes Humanos en Investigación del Recinto de Ciencias Médicas (UPR MSC IRB) de la Universidad de Puerto Rico. Ellos vigilarán como se está llevando a cabo el estudio y revisarán su información para estos propósitos.

La información brindada podría ser revisada por la Oficina Para la Protección de Participantes Humanos en Investigación del Recinto de Ciencias Médicas (UPR MSC IRB). Ellos son un grupo de personas que llevan a cabo revisiones independientes de las investigaciones como requeridas por las agencias reguladoras.

Se harán todos los esfuerzos posibles para proteger su identidad en este estudio. Cualquier información que se obtenga en relación con este estudio que pueda identificarlo permanecerá confidencial según lo requiere la ley. Las regulaciones federales proveen garantías de la privacidad, seguridad y el acceso autorizado a su información. Es nuestra prioridad el proteger la información personal que lo pueda identificar, para esto se tomarán las siguientes medidas:

Toda la información recopilada que lo identifique se guardará bajo llave en archivos. A cada participante de este estudio se le asigna un número de estudio para evitar utilizar otros números que pudieran identificarlo, tales como el seguro social o el número de licencia de conducir. Los resultados del estudio serán analizados mediante el uso de este número solamente y no se identificará a ningún individuo en las publicaciones relacionadas al estudio.

Usted podría cancelar esta autorización en cualquier momento enviando una nota escrita a uno de los investigadores a la siguiente dirección:

**Dra. Glorisa Canino, PhD**
Instituto de Investigación de
Ciencias de la Conducta
Universidad de Puerto Rico
Recinto de Ciencias Médicas

**Velda J. González, RN**
Centro de Cáncer
Universidad de Puerto Rico
PMB-371 Po Box 70344
San Juan, PR 00936-8344

**IRB APPROVED** (MSC/UPR)
From 04/24/14 TO 04/24/15

PO Box 365067
San Juan, PR, 00936
(787) 754-8624
Correo electrónico: glorida.canino@upr.edu

(787) 772-8300 ext. 1129
Correo electrónico:
velda.gonzalez@upr.edu

Si usted cancela esta autorización, el investigador principal no utilizará más o revelará su información de salud bajo la autorización de este estudio, a menos que él/ella necesite usar o revelar alguna de su información de salud para preservar la integridad científica del estudio. Información suministrada antes de usted cancelar esta autorización podría continuar utilizándose por los investigadores.

La autorización para usar y divulgar información protegida de salud para propósitos de investigación es completamente voluntaria. Sin embargo, si usted no firma este documento usted no podrá participar en este estudio.

**¿QUE COMPENSACION PUEDO RECIBIR EN CASO DE DAÑO POR PARTICIPAR ESTE ESTUDIO?**
En el caso de lesión física y/o mental como resultado de este estudio de investigación, usted recibirá tratamiento médico libre de costo en el Hospital Universitario de la Universidad o cualquier otro hospital designado por el Rector del Recinto de Ciencias Médicas de la Universidad de Puerto Rico. La Universidad de Puerto Rico no ofrecerá ninguna forma de remuneración directamente a usted. Sin embargo, firmando esta forma de consentimiento usted no renunciará a cualquier derecho legal.

**¿QUIEN PROVEE FONDOS PARA ESTE ESTUDIO?**
Este estudio no recibe fondos.

**¿A QUIEN DEBO LLAMAR SI TENGO PREGUNTAS SOBRE ESTE ESTUDIO?**
Si usted tiene alguna pregunta sobre este estudio o sobre su participación en este estudio puede comunicarse con la Dra. Glorisa Canino al (787) 754-8624 o con Velda González al (787) 772-8300 extensión 1129 o al teléfono 787-457-8508 fuera de horas laborables. De no encontrarse disponible, usted debe dejar un mensaje en la grabadora telefónica y se le devolverá la llamada rápidamente.

Si usted tiene alguna pregunta sobre sus derechos como participante del estudio, usted puede contactar a la:

Oficina de Protección de Participantes Humanos en Investigación.
Teléfono (787) 758-2525 ext. 2510 ó 2511
Correo electrónico: opphi.rcm@upr.edu

IRB APPROVED (MSC/UPR)
From 04/24/14 TO 04/24/15

No firme este consentimiento a menos que haya tenido la oportunidad de hacer preguntas y haya recibido respuestas satisfactorias a todas sus preguntas.

Se le entregará una copia de este consentimiento con el sello de aprobación del IRB del Recinto de Ciencias Médicas.

**CONSENTIMIENTO INFORMADO**

Yo he leído (o me han leído) la información anterior sobre el estudio "Expresión de genes y síntoma de fatiga (cansancio) en hombres Puertorriqueños" y se me ha explicado su contenido. Me han dado la oportunidad de hacer preguntas. Todas mis preguntas han sido contestadas. Libremente doy mi consentimiento para participar en este estudio. Autorizo el uso y divulgación de mi información de salud a las agencias mencionadas en la sección de autorizaciones de este consentimiento para los propósitos ya descritos. Por firmar este consentimiento informado no he renunciado a ninguno de mis derechos legales.

____________________________
Nombre del participante

____________________________ __________________
Firma del participante Fecha de la firma

| Nombre del investigador<br>o persona designada que<br>obtiene el consentimiento informado | Firma | Fecha de la firma |
|---|---|---|

IRB APPROVED (MSC/UPR)
From 04/24/14 TO 04/04/15

**Appendix H**

Demographic Form (English version)

Age: ______years

Marital Status:

| | | | |
|---|---|---|---|
| Married | ______ | (1) | Number of Children:_______ |
| Single | ______ | (2) | |
| Widowed | ______ | (3) | |
| Divorced | ______ | (4) | |
| Living with Partner | ______ | (5) | |
| Other | ______ | (6) | |

Religion:

| | | |
|---|---|---|
| Protestant | ______ | (0) |
| Catholic | ______ | (1) |
| Jewish | ______ | (2) |
| Buddhist | ______ | (3) |
| Other | ______ | (4) |

Ethnic Background (check all that apply):

| | | |
|---|---|---|
| White | ______ | (1) |
| African American/Black | ______ | (2) |
| Mix of races | ______ | (3) |

Occupation or Job: ____________________________________________

Place of Employment (if employed): ______________________________

☐ Full Time ☐ Part Time

Highest Education Completed by [Respondent]

☐ Not attended school
☐ Elementary or middle school
☐ Some high school
☐ High school diploma or equivalent (GED)
☐ Some higher education, no degree
☐ Technical or vocational school
☐ Associate degree
☐ BS/BA
☐ Graduate degree

**Appendix H**

Also, give number of years in school: ______years
Primary caregiver at home and relationship: ________________________________

If married, spouse's age: ________ years.

Spouse: Highest Education Completed
- ☐ Not attended school
- ☐ Elementary or middle school
- ☐ Some high school
- ☐ High school diploma or equivalent (GED)
- ☐ Some higher education, no degree
- ☐ Technical or vocational school
- ☐ Associate degree
- ☐ BS/BA
- ☐ Graduate degree

THANK YOU VERY MUCH FOR COMPLETING THE QUESTIONNAIRE

**Appendix I**

Demographic Form (Spanish version)

Edad: ______años

Estatus Marital:

| | | | |
|---|---|---|---|
| Casado | _______ | (1) | Número de hijos:_______ |
| Soltero | _______ | (2) | |
| Viudo | _______ | (3) | |
| Divorciado | _______ | (4) | |
| Convive | _______ | (5) | |
| Otro | _______ | (6) | |

Religión:

| | | |
|---|---|---|
| Protestante | _______ | (0) |
| Católico | _______ | (1) |
| Judío | _______ | (2) |
| Budista | _______ | (3) |
| Otra | _______ | (4) |

RaícesÉtnicas:

| | | |
|---|---|---|
| Blanco | _______ | (1) |
| Afro Americano/Negro | _______ | (2) |
| Mescla de razas | _______ | (3) |

Ocupación: _____________________________________________

Lugar de Trabajo: _______________________________

☐ Tiempo Completo ☐ Tiempo Parcial

Grado másalto completado
- ☐ No atendió escuela
- ☐ Elemental o intermedia
- ☐ Algo de superior
- ☐ Escuela Superior o equivalente (GED)
- ☐ Mayoreducación pero sin grado
- ☐ Técnica o vocacional
- ☐ Grado Asociado
- ☐ Bachillerato
- ☐ Maestría o doctorado

**Appendix I**

Total de número de años en escuela: ______años
Cuidador principal: ________________________________

Si casado, edad de conyugue: ________ años.

Esposa: Grado másalto Completado
- ☐ No atendió escuela
- ☐ Elemental o intermedia
- ☐ Algo de superior
- ☐ Escuela Superior o equivalente (GED)
- ☐ Mayoreducación pero sin grado
- ☐ Técnica o vocacional
- ☐ Grado Asociado
- ☐ Bachillerato
- ☐ Maestría o doctorado

Total de número de años en escuela: ______años

GRACIAS POR COMPLETAR EL CUESTIONARIO

**Appendix J**

## FACIT Fatigue Scale (Version 4)

English (Universal) 16 November 2007


Page 1 of 1

Below is a list of statements that other people with your illness have said are important**. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

| | Not at all | A little bit | Somewhat | Quite a bit | Very much |
|---|---|---|---|---|---|
| I feel fatigued………….................................... | 0 | 1 | 2 | 3 | 4 |
| I feel weak all over........................................... | 0 | 1 | 2 | 3 | 4 |
| I feel listless ("washed out")…......................... | 0 | 1 | 2 | 3 | 4 |
| I feel tired.......................................................... | 0 | 1 | 2 | 3 | 4 |
| I have trouble starting things because I am tired.................................................................... | 0 | 1 | 2 | 3 | 4 |
| I have trouble finishing things because I am tired..................................................................... | 0 | 1 | 2 | 3 | 4 |
| I have energy...................................................... | 0 | 1 | 2 | 3 | 4 |
| I am able to do my usual activities..................... | 0 | 1 | 2 | 3 | 4 |
| I need to sleep during the day............................ | 0 | 1 | 2 | 3 | 4 |
| I am too tired to eat........................................... | 0 | 1 | 2 | 3 | 4 |
| I need help doing my usual activities................. | 0 | 1 | 2 | 3 | 4 |
| I am frustrated by being too tired to do the things I want to do............................................. | 0 | 1 | 2 | 3 | 4 |
| I have to limit my social activity because I am tired...................................................................... | 0 | 1 | 2 | 3 | 4 |

**Appendix K**

## FACIT-F (4a Versión)

Spanish (Universal) 18 June 2012



Página 1de 1

**Marque un solo número por línea para indicar la respuesta que corresponde a los últimos 7 días.**

| | Nada | Un poco | Algo | Mucho | Muchísimo |
|---|---|---|---|---|---|
| Me siento agotado.............................................. | 0 | 1 | 2 | 3 | 4 |
| Siento debilidad en todo el cuerpo....................... | 0 | 1 | 2 | 3 | 4 |
| Me siento decaído.............................................. | 0 | 1 | 2 | 3 | 4 |
| Me siento cansado.............................................. | 0 | 1 | 2 | 3 | 4 |
| Tengo dificultad para comenzar las cosas porque estoycansado..................................................... | 0 | 1 | 2 | 3 | 4 |
| Tengo dificultad para terminar las cosas porque estoycansado..................................................... | 0 | 1 | 2 | 3 | 4 |
| Tengo energía..................................................... | 0 | 1 | 2 | 3 | 4 |
| Soy capaz de hacer mis actividades habituales (ej. trabajar, ir a la escuela, hacer compras)......... | 0 | 1 | 2 | 3 | 4 |
| Necesito dormir durante el día............................ | 0 | 1 | 2 | 3 | 4 |
| Estoy demasiado cansado para comer................. | 0 | 1 | 2 | 3 | 4 |
| Necesito ayuda para hacer mis actividades habituales............................................................ | 0 | 1 | 2 | 3 | 4 |
| Estoy frustrado porque estoy demasiado cansado para hacer las cosas que quiero hacer................. | 0 | 1 | 2 | 3 | 4 |
| Tengo que limitar mis actividades sociales debido al cansancio............................................ | 0 | 1 | 2 | 3 | 4 |

## Appendix L

### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

**INSTRINSTRUCTIONS:** We are interested in finding out about the kinds of physical activities that people do as part of their dally everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical

activities like heavy lifting, digging, aerobics, or fast bicycling?

**_____ days per week**

No vigorous physical activities ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

**_____ hours per day**

**_____ minutes per day _____**Don’t know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate**

activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical

activities like carrying light loads, bicycling at a regular pace or double tennis?

Do not include walking.

**_____ days per week**

No moderate physical activities ***Skip to question 5***

Appendix L

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

______Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

______Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

_____ Don't know/Not sure

# Appendix M

## IPAQ

## CUESTIONARIO INTERNACIONAL DE ACTIVIDAD FÍSICA

**Instrucciones:** Estamos interesados en saber acerca de la clase de actividad física que la gente hace como parte de su vida diaria. Las preguntas se referirán acerca del tiempo que usted utilizó siendo físicamente activo(a) en los **últimos 7 días**. Por favor responda cada pregunta aún si usted no se considera una persona activa. Por favor piense en aquellas actividades que usted hace como parte del trabajo, en el jardín y en la casa, para ir de un sitio a otro, y en su tiempo libre de descanso, ejercicio o deporte.

Piense acerca de todas aquellas actividades **vigorosas** que usted realizó en los **últimos 7 días**. Actividades **vigorosas** son las que requieren un esfuerzo físico fuerte y le hacen respirar mucho más fuerte que lo normal. Piense *solamente* en esas actividades que usted hizo por lo menos 10 minutos continuos.

Durante los **últimos 7 días**, ¿Cuántos días realizó usted actividades físicas **vigorosas** como levantar objetos pesados, excavar, aeróbicos, o pedalear rápido en bicicleta?

_____ **días por semana**

Ninguna actividad física vigorosa → ***Pase a la pregunta 3***

¿Cuánto tiempo en total usualmente le tomó realizar actividades físicas **vigorosas** en uno de esos días que las realizó?

_____ **horas por día**

_____ **minutos por día**

______ No sabe/No está seguro(a)

Piense acerca de todas aquellas actividades **moderadas** que usted realizo en los **últimos 7 días.** Actividades **moderadas** son aquellas que requieren un esfuerzo físico moderado y le hace respirar algo más fuerte que lo normal. Piense *solamente* en esas actividades que usted hizo por lo menos 10 minutos continuos.

Durante los **últimos 7 días**, ¿Cuántos días hizo usted actividades físicas **moderadas** tal como cargar objetos livianos, pedalear en bicicleta a paso regular, o jugar dobles de tenis? No incluyacaminatas.

_____ **díasporsemana**

Ninguna actvidad física moderada → ***Pase a la pregunta 5***

Usualmente, ¿Cuánto tiempo dedica usted en uno de esos días haciendo actividades físicas **moderadas**?

_____ **horas por día**

_____ **minutos por día**

No sabe/No está seguro(a)

Piense acerca del tiempo que usted dedicó a caminar en los **últimos 7 días**. Esto incluye trabajo en la casa, caminatas para ir de un sitio a otro, o cualquier otra caminata que usted hizo únicamente por recreación, deporte, ejercicio, o placer.

5. Durante los **últimos 7 días**, ¿Cuántos días caminó usted por al menos 10 minutos continuos?

_____ **días por semana**

No caminó → ***Pase a la pregunta 7***

Usualmente, ¿Cuánto tiempo gastó usted en uno de esos días **caminando**?

_____ **horas por día**

_____ **minutos por día**

No sabe/No está seguro(a)

La última pregunta se refiere al tiempo que usted permanenció**sentado(a)** en la semana en los **últimos 7 días**. Incluya el tiempo sentado(a) en el trabajo, la casa, estudiando, y en su tiempo libre. Esto puede incluir tiempo sentado(a) en un escritorio, visitando amigos(as), leyendo o permanecer sentado(a) o acostado(a) mirando televisión.

Durante los **últimos 7 días**, ¿Cuánto tiempo permaneció **sentado(a)** en un **día en la semana**?

_____ **horas por día**

_____ **minutos por día**

No sabe/No está seguro(a)

**Appendix N**

PROMIS Item Bank v. 1.0 – Sleep Disturbance – Short Form 8b
Spanish (Universal) 15 August 2012 © 2008-2012 PROMIS Health Organization and PROMIS Cooperative Group Page 1 of 1

**Responda a cada enunciado marcando una casilla por línea.**

| **En los últimos 7 días...** | Nada | Un Poco | Algo | Mucho | Muchísimo |
|---|---|---|---|---|---|
| Tuve el sueño inquieto............................... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| Me sentí satisfecho/a con mi sueño........... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| Mi sueño fuereparador.............................. | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| Tuve dificultad para dormirme................... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

| **En los últimos 7 días...** | Nada | Un Poco | Algo | Mucho | Muchísimo |
|---|---|---|---|---|---|
| Tuve problemas para permanecer dormido..... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| Dormí mal...................................................... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| Dormí suficiente............................................ | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

| **En los últimos 7 días...** | Muy mala | Mala | Pasable | Buena | Muybuena |
|---|---|---|---|---|---|
| La calidad de mi sueño fue.......................... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

**Appendix O**

PROMIS Item Bank v. 1.0 – Sleep Disturbance – Short Form 8b (Universal) 15 August 2012 © 2008-2012 PROMIS Health Organization and PROMIS Cooperative Group Page 1 of 1

**Please respond to each item by marking one box per now.**

| **In the past 7 days...** | Not at all | A little bit | Somewhat | Quite a bit | Very much |
|---|---|---|---|---|---|
| My sleep was restless................................ | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |
| I was satisfied with my sleep...................... | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |
| My sleep was refreshing............................ | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |
| I had difficulty falling asleep...................... | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |

| **In the past 7 days...** | Not at all | A little bit | Somewhat | Quite a bit | Very much |
|---|---|---|---|---|---|
| I had trouble staying asleep.......................... | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |
| I had trouble sleeping................................... | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |
| I got enough sleep......................................... | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |

| **In the past 7 days...** | Very poor | Poor | Fair | Good | Very good |
|---|---|---|---|---|---|
| My sleep quality was................................... | □ 1 | □ 2 | □ 3 | □ 4 | □ 5 |

## Appendix P

The Hamilton Rating Scale for Depression

| Item | Value |
|---|---|
| **DEPRESSED MOOD** (*Sadness, hopeless, helpless, worthless*) | Absent<br>These feelings are indicated only on questioning<br>These feelings are spontaneously reported verbally<br>Communicates feelings non-verbally i.e., through facial expression, posture, voice, and tendency to weep<br>Patient reports VIRTUALLY ONLY these feelings in his spontaneous verbal and non-verbal communication |
| **FEELINGS OF GUILT** | Absent<br>Self-reproach, feels he has let people down<br>Ideas of guilt or rumination over past errors or sinful deed<br>Present illness is a punishment. Delusions of guilt<br>Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations |
| **SUICIDE** | Absent<br>Feels life is not worth living<br>Wishes he were dead or any thoughts of possible death to self<br>Suicide ideas or gesture<br>Attempts at suicide (any serious attempt rates) |
| **INSOMNIA EARLY** | No difficulty falling asleep<br>Complains of occasional difficulty falling asleep - more than 1/2 hour<br>Complains of nightly difficulty falling asleep |
| **INSOMNIA MIDDLE** | No difficulty<br>Patient complains of being restless and disturbed during the night<br>Waking during the night - any getting out of bed (except for purposes of voiding) |
| **INSOMNIA LATE** | No difficulty<br>Waking in early hours of the morning but goes back to sleep<br>Unable to fall asleep again if he gets out of bed |

# Appendix P



| | |
|---|---|
| **WORK AND ACTIVITIES** | No difficulty<br>Thoughts and feelings of incapacity, fatigue or weakness related to activities (work or hobbies)<br>Loss of interest in activities (hobbies or work) - either directly reported by patient, or indirectly in listlessness, indecision and vacillation (feels he has to push himself to work or do activities)<br>Decrease in actual time spent in activities or decrease in productivity.<br>In hospital, if patient does not spend at least three hours a day in activities (hospital job or hobbies) exclusive of ward chores<br>Stopped working because of present illness. In hospital, if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted |
| **RETARDATION: PSYCHOMOTOR** (Slowness of thought and speech; impaired ability to concentrate; decreased motor activity) | Normal speech and thought1.<br>Slight retardation at interview<br>Obvious retardation at interview<br>Interview difficult<br>Complete stupor |
| **AGITATION** | None<br>Fidgetiness<br>Playing with hands, hair, etc.<br>Moving about, can't sit still<br>Hand wringing, nail biting, hair-pulling, biting of lips |
| **ANXIETY: PSYCHIC** | No difficulty<br>Subjective tension and irritability<br>Worrying about minor matters<br>Apprehensive attitude apparent in face or speech<br>Fears expressed without questioning |
| **ANXIETY: SOMATIC** (Physiological concomitants of anxiety, such as - Gastro-intestinal: dry mouth, wind, indigestion, diarrhea, cramps, belching. - Cardio-vascular: palpitations, headaches. - Respiratory: hyperventilation, sighing. - Urinary frequency - Sweating) | Absent<br>Mild<br>Moderate<br>Severe<br>Incapacitating<br>**Appendix O** |
| **SOMATIC SYMPTOMS: GASTROINTESTINAL** | None<br>Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen<br>Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for gastro-intestinal symptoms |

| | |
|---|---|
| **SOMATIC SYMPTOMS: GENERAL** | None<br>Heaviness in limbs, back or head. Backaches, headache, muscle aches. Loss of energy and fatigability<br>Any clear-cut symptom |
| **GENITAL SYMPTOMS** (loss of libido, menstrual disturbances) | Absent<br>Mild<br>Severe |
| **HYPOCHONDRIASIS** | Not present<br>Self-absorption (bodily)<br>Preoccupation with health<br>Frequent complaints, requests for help, etc...<br>Hypochondriacal delusions |
| **LOSS OF WEIGHT** | No weight loss<br>Probable weight loss associated with present illness (>500g/week)<br>Definite weight loss(>1kg/week) |
| **INSIGHT** | Not depressed (based on above items) OR Acknowledges being depressed and ill<br>Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.<br>Denies being ill at all |

Total ___________

**Appendix Q. Health Form**

ID# ________________ | Age: ________________ | Evaluation Date: ________________

RT Simulation Date: ________________________________________

STAGE OF DISEASE:______

Gleason score:_________

Type of ADT, ________ and duration ___________

# of RT fractions ________ # of RT fields__________

Pelvic RT, yes or no: ________

Weight ___________ Height________________

COMORBID CONDITIONS:

Current medications:

________________________________
________________________________
________________________________
________________________________
________________________________
________________________________
________________________________
________________________________
________________________________
________________________________
.

| Check if it applies: | | | Baseline | Midpoint | EOT |
|---|---|---|---|---|---|
| HTN | ☐ | PSA | | | |
| Diabetes | ☐ | Hgb | | | |
| Anemia | ☐ | Albumin | | | |
| Heart Attack | ☐ | Thyroxine | | | |
| Depression | ☐ | Interruption of RT | | | |
| Anxiety | ☐ | Hx. of infection | | | |
| Thyroid problems | ☐ | ER visits | | | |
| Other: | ☐ | Hospital Admission | | | |
| | | Weight | | | |

# Appendix R. Genes with a FDR <0.01

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 201737_s_at | 6-Mar | membrane-associated ring finger (C3HC4) 6, E3 ubiquitin protein ligase | -0.416483365 | 4.14E-05 | 0.008035648 |
| 213666_at | 6-Sep | septin 6 | -0.541015854 | 8.23E-06 | 0.006439647 |
| 214298_x_at | 6-Sep | septin 6 | -0.547234599 | 1.97E-05 | 0.006791288 |
| 1555526_a_at | 6-Sep | septin 6 | -0.41827274 | 4.01E-05 | 0.007932185 |
| 200965_s_at | ABLIM1 | actin binding LIM protein 1 | -0.675395949 | 3.74E-06 | 0.005447157 |
| 210461_s_at | ABLIM1 | actin binding LIM protein 1 | -0.422225223 | 0.000103059 | 0.010514226 |
| 205213_at | ACAP1 | ArfGAP with coiled-coil, ankyrin repeat and PH domains 1 | -0.355740857 | 0.000108657 | 0.010704185 |
| 205377_s_at | ACHE | acetylcholinesterase (Yt blood group) | 0.246436535 | 4.09E-05 | 0.007970846 |
| 205997_at | ADAM28 | ADAM metallopeptidase domain 28 | -0.568128942 | 6.47E-05 | 0.009166579 |
| 1553427_at | ADAMTS15 | ADAM metallopeptidase with thrombospondin type 1 motif, 15 | 0.263574775 | 5.36E-05 | 0.008497204 |
| 203865_s_at | ADARB1 | adenosine deaminase, RNA-specific, B1 | -0.594770997 | 3.20E-06 | 0.005447157 |
| 209979_at | ADARB1 | adenosine deaminase, RNA-specific, B1 | 0.41613665 | 2.00E-05 | 0.006791288 |
| 201752_s_at | ADD3 | adducin 3 (gamma) | -0.437491737 | 9.06E-05 | 0.010132739 |
| 220606_s_at | ADPRM | ADP-ribose/CDP-alcohol diphosphatase, manganese-dependent | -0.477460006 | 1.62E-05 | 0.006523888 |
| 219361_s_at | AEN | apoptosis enhancing nuclease | 0.330497035 | 7.99E-07 | 0.003642121 |
| 217729_s_at | AES | amino-terminal enhancer of split | -0.410526942 | 0.000114131 | 0.010851164 |
| 227198_at | AFF3 | AF4/FMR2 family, member 3 | -0.822274258 | 4.92E-05 | 0.00833877 |
| 1552287_s_at | AFG3L1P | AFG3-like AAA ATPase 1, pseudogene | -0.555411818 | 5.01E-05 | 0.008348442 |
| 235926_at | ANAPC5 | anaphase promoting complex subunit 5 | -0.605493188 | 5.64E-05 | 0.008735693 |
| 212583_at | AQR | aquarius intron-binding spliceosomal factor | -0.218554687 | 6.11E-05 | 0.009021899 |
| 213039_at | ARHGEF18 | Rho/Rac guanine nucleotide exchange factor (GEF) 18 | -0.412735266 | 7.51E-05 | 0.00943974 |
| 242239_at | ARL5B-AS1 | ARL5B antisense RNA 1 | -0.531037765 | 0.000116397 | 0.010972383 |
| 205047_s_at | ASNS | asparagine synthetase (glutamine-hydrolyzing) | -0.676695703 | 1.70E-05 | 0.006523888 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 209693_at | ASTN2 | astrotactin 2 | 0.237321784 | 7.82E-05 | 0.009573853 |
| 232838_at | ASXL3 | additional sex combs like 3 (Drosophila) | 0.280291969 | 4.55E-05 | 0.008171695 |
| 227365_at | ATCAY | ataxia, cerebellar, Cayman type | 0.275145014 | 9.27E-05 | 0.010202143 |
| 231825_x_at | ATF7IP | activating transcription factor 7 interacting protein | -0.407397447 | 2.39E-05 | 0.007221094 |
| 210858_x_at | ATM | ataxia telangiectasia mutated | -0.472514912 | 1.96E-05 | 0.006791288 |
| 208442_s_at | ATM | ataxia telangiectasia mutated | -0.510325909 | 4.31E-05 | 0.008151598 |
| 223339_at | ATPIF1 | ATPase inhibitory factor 1 | -0.273887652 | 8.61E-05 | 0.009846679 |
| 204516_at | ATXN7 | ataxin 7 | -0.412712592 | 0.000101383 | 0.010514226 |
| 221234_s_at | BACH2 | BTB and CNC homology 1, basic leucine zipper transcription factor 2 | -0.989631763 | 4.86E-07 | 0.003632198 |
| 227173_s_at | BACH2 | BTB and CNC homology 1, basic leucine zipper transcription factor 2 | -0.468213388 | 5.53E-06 | 0.00592514 |
| 219667_s_at | BANK1 | B-cell scaffold protein with ankyrin repeats 1 | -1.039378218 | 3.81E-05 | 0.007932185 |
| 1558662_s_at | BANK1 | B-cell scaffold protein with ankyrin repeats 1 | -0.702967876 | 4.96E-05 | 0.00833877 |
| 223134_at | BBX | bobby sox homolog (Drosophila) | -0.457352353 | 9.91E-05 | 0.010461606 |
| 222891_s_at | BCL11A | B-cell CLL/lymphoma 11A (zinc finger protein) | -0.60307787 | 5.55E-05 | 0.008663178 |
| 222895_s_at | BCL11B | B-cell CLL/lymphoma 11B (zinc finger protein) | -0.68569958 | 1.59E-05 | 0.006523888 |
| 219528_s_at | BCL11B | B-cell CLL/lymphoma 11B (zinc finger protein) | -0.695201346 | 3.33E-05 | 0.007844053 |
| 201261_x_at | BGN | biglycan | 0.305545609 | 7.26E-05 | 0.009255079 |
| 202931_x_at | BIN1 | bridging integrator 1 | -0.294464341 | 3.78E-05 | 0.007932185 |
| 214439_x_at | BIN1 | bridging integrator 1 | -0.327577901 | 4.56E-05 | 0.008171695 |
| 207655_s_at | BLNK | B-cell linker | -0.844816248 | 3.74E-05 | 0.007932185 |
| 207186_s_at | BPTF | bromodomain PHD finger transcription factor | -0.458313139 | 3.50E-06 | 0.005447157 |
| 208685_x_at | BRD2 | bromodomain containing 2 | -0.376609434 | 9.81E-05 | 0.010434823 |
| 215010_s_at | BRSK2 | BR serine/threonine kinase 2 | 0.371996264 | 3.85E-05 | 0.007932185 |
| 229827_at | BUB3 | BUB3 mitotic checkpoint protein | 0.246540068 | 8.21E-05 | 0.009761164 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 238593_at | C11orf80 | chromosome 11 open reading frame 80 | -0.58521126 | 8.08E-05 | 0.009698499 |
| 1559097_at | C14orf64 | chromosome 14 open reading frame 64 | -0.486999329 | 6.36E-06 | 0.006034078 |
| 228666_at | C15orf38 | chromosome 15 open reading frame 38 | 0.264286308 | 4.10E-05 | 0.007970846 |
| 231153_at | C16orf86 | chromosome 16 open reading frame 86 | 0.277535825 | 9.00E-05 | 0.010123501 |
| 1557828_a_at | C5orf28 | chromosome 5 open reading frame 28 | -0.564640857 | 7.12E-05 | 0.009212663 |
| 215954_s_at | CACTIN | cactin, spliceosome C complex subunit | -0.289637725 | 0.000112994 | 0.010834971 |
| 219896_at | CALY | calcyon neuron-specific vesicular protein | 0.265848172 | 9.06E-05 | 0.010132739 |
| 229029_at | CAMK4 | calcium/calmodulin-dependent protein kinase IV | -0.569849314 | 3.68E-05 | 0.007932185 |
| 231710_at | CAPS | calcyphosine | 0.400140237 | 9.53E-05 | 0.010312924 |
| 231862_at | CBX5 | chromobox homolog 5 | -0.336978513 | 4.69E-05 | 0.008234897 |
| 239014_at | CCAR1 | cell division cycle and apoptosis regulator 1 | -0.490146901 | 1.57E-05 | 0.006523888 |
| 237475_x_at | CCDC152 | coiled-coil domain containing 152 | -0.40267085 | 5.48E-05 | 0.00860224 |
| 206337_at | CCR7 | chemokine (C-C motif) receptor 7 | -0.855626052 | 1.27E-05 | 0.006492519 |
| 206508_at | CD70 | CD70 molecule | 0.288242775 | 8.66E-06 | 0.006492519 |
| 215925_s_at | CD72 | CD72 molecule | -0.587775424 | 5.68E-05 | 0.008735693 |
| 1555779_a_at | CD79A | CD79a molecule, immunoglobulin-associated alpha | -0.648004243 | 7.94E-07 | 0.003642121 |
| 205049_s_at | CD79A | CD79a molecule, immunoglobulin-associated alpha | -0.768059007 | 3.12E-05 | 0.007762527 |
| 205297_s_at | CD79B | CD79b molecule, immunoglobulin-associated beta | -0.446891887 | 0.000113494 | 0.010834971 |
| 203794_at | CDC42BPA | CDC42 binding protein kinase alpha (DMPK-like) | 0.215974634 | 0.00010241 | 0.010514226 |
| 215181_at | CDH22 | cadherin 22, type 2 | 0.340165492 | 1.66E-05 | 0.006523888 |
| 206575_at | CDKL5 | cyclin-dependent kinase-like 5 | 0.319115759 | 6.97E-05 | 0.009195279 |
| 240889_at | CDRT15L2 | CMT1A duplicated region transcript 15-like 2 | 0.316506378 | 4.38E-05 | 0.008171695 |
| 228868_x_at | CDT1 | chromatin licensing and DNA replication factor 1 | 0.365857616 | 3.98E-05 | 0.007932185 |
| 209489_at | CELF1 | CUGBP, Elav-like family member 1 | -0.297604956 | 7.04E-05 | 0.009212663 |
| 207331_at | CENPF | centromere protein F, 350/400kDa | 0.170722501 | 8.02E-05 | 0.009694254 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 239442_at | CEP68 | centrosomal protein 68kDa | -0.332895351 | 0.000111611 | 0.010789763 |
| 206824_at | CES1P1 | carboxylesterase 1 pseudogene 1 | 0.350463495 | 8.40E-05 | 0.009786493 |
| 234706_x_at | CFTR | cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7) | 0.126591832 | 0.000102843 | 0.010514226 |
| 225026_at | CHD6 | chromodomain helicase DNA binding protein 6 | -0.38228634 | 4.03E-05 | 0.007932185 |
| 212313_at | CHMP7 | charged multivesicular body protein 7 | -0.407111494 | 1.18E-06 | 0.004276757 |
| 213628_at | CLCC1 | chloride channel CLIC-like 1 | -0.329268827 | 1.70E-05 | 0.006523888 |
| 234443_at | CLSTN2-AS1 | CLSTN2 antisense RNA 1 | 0.257562281 | 3.81E-05 | 0.007932185 |
| 231884_at | CNTROB | centrobin, centrosomal BRCA2 interacting protein | 0.326559424 | 1.50E-05 | 0.006523888 |
| 232733_s_at | COL20A1 | collagen, type XX, alpha 1 | 0.267559014 | 3.20E-05 | 0.007775372 |
| 217484_at | CR1 | complement component (3b/4b) receptor 1 (Knops blood group) | 0.2406621 | 0.000109809 | 0.010778823 |
| 218648_at | CRTC3 | CREB regulated transcription coactivator 3 | -0.402085296 | 1.43E-05 | 0.006492519 |
| 207030_s_at | CSRP2 | cysteine and glycine-rich protein 2 | 0.227633344 | 1.76E-06 | 0.004576131 |
| 235523_at | CTC1 | CTS telomere maintenance complex component 1 | -0.357302929 | 2.06E-05 | 0.006799529 |
| 222819_at | CTPS2 | CTP synthase 2 | -0.290947316 | 1.62E-05 | 0.006523888 |
| 200838_at | CTSB | cathepsin B | 0.317453237 | 9.17E-05 | 0.010159282 |
| 224703_at | DCAF5 | DDB1 and CUL4 associated factor 5 | -0.307448623 | 6.65E-05 | 0.009187381 |
| 203409_at | DDB2 | damage-specific DNA binding protein 2, 48kDa | 0.350384304 | 3.20E-05 | 0.007775372 |
| 200694_s_at | DDX24 | DEAD (Asp-Glu-Ala-Asp) box helicase 24 | -0.369252469 | 7.14E-05 | 0.009212663 |
| 1568815_a_at | DDX50 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 50 | -0.524185446 | 1.52E-05 | 0.006523888 |
| 221081_s_at | DENND2D | DENN/MADD domain containing 2D | -0.500251911 | 0.000101528 | 0.010514226 |
| 226116_at | DFFA | DNA fragmentation factor, 45kDa, alpha polypeptide | -0.399748384 | 8.10E-05 | 0.009698499 |
| 215003_at | DGCR9 | DiGeorge syndrome critical region gene 9 | 0.261777148 | 1.14E-05 | 0.006492519 |
| 203385_at | DGKA | diacylglycerol kinase, alpha 80kDa | -0.388542741 | 1.07E-05 | 0.006492519 |
| 211272_s_at | DGKA | diacylglycerol kinase, alpha 80kDa | -0.41120375 | 1.16E-05 | 0.006492519 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 214193_s_at | DIEXF | digestive organ expansion factor homolog (zebrafish) | -0.499589308 | 0.000112091 | 0.010789763 |
| 215529_x_at | DIP2A | DIP2 disco-interacting protein 2 homolog A (Drosophila) | -0.364417667 | 0.000109006 | 0.010719202 |
| 244725_at | DMRTA1 | DMRT-like family A1 | 0.313519975 | 4.48E-05 | 0.008171695 |
| 1554078_s_at | DNAJA3 | DnaJ (Hsp40) homolog, subfamily A, member 3 | -0.312312494 | 8.89E-05 | 0.010058626 |
| 232874_at | DOCK9 | dedicator of cytokinesis 9 | -0.452709825 | 4.00E-05 | 0.007932185 |
| 226009_at | DPCD | deleted in primary ciliary dyskinesia homolog (mouse) | 0.441423673 | 1.32E-05 | 0.006492519 |
| 209391_at | DPM2 | dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit | 0.457599025 | 0.000100451 | 0.010514226 |
| 217671_at | DSERG1 | Down syndrome encephalopathy related protein 1 | -0.231796963 | 6.17E-05 | 0.009050415 |
| 239733_at | DYDC2 | DPY30 domain containing 2 | 0.188935599 | 4.98E-05 | 0.008348442 |
| 221586_s_at | E2F5 | E2F transcription factor 5, p130-binding | -0.424440392 | 6.52E-05 | 0.009173198 |
| 233261_at | EBF1 | early B-cell factor 1 | -0.4289617 | 8.10E-06 | 0.006439647 |
| 227646_at | EBF1 | early B-cell factor 1 | -0.846448213 | 2.56E-05 | 0.007379223 |
| 238761_at | ELK4 | ELK4, ETS-domain protein (SRF accessory protein 1) | -0.476826864 | 1.34E-05 | 0.006492519 |
| 228674_s_at | EML4 | echinoderm microtubule associated protein like 4 | -0.351981709 | 0.000111395 | 0.010789763 |
| 219912_s_at | ENPP3 | ectonucleotide pyrophosphatase/phosphodiesterase 3 | 0.273238975 | 7.00E-05 | 0.009195279 |
| 212375_at | EP400 | E1A binding protein p400 | -0.41526683 | 5.27E-05 | 0.008481109 |
| 204718_at | EPHB6 | EPH receptor B6 | -0.237230285 | 2.81E-05 | 0.007549101 |
| 211603_s_at | ETV4 | ets variant 4 | 0.367180598 | 1.58E-05 | 0.006523888 |
| 244500_s_at | EVI5L | ecotropic viral integration site 5-like | 0.28410611 | 0.000112475 | 0.010807656 |
| 207541_s_at | EXOSC10 | exosome component 10 | -0.35911111 | 8.56E-05 | 0.00983175 |
| 217234_s_at | EZR | ezrin | -0.438680772 | 8.30E-05 | 0.009786493 |
| 208623_s_at | EZR | ezrin | -0.497262521 | 9.61E-05 | 0.010342213 |
| 202862_at | FAH | fumarylacetoacetate hydrolase (fumarylacetoacetase) | 0.30366866 | 4.55E-05 | 0.008171695 |
| 221602_s_at | FAIM3 | Fas apoptotic inhibitory molecule 3 | -0.649478155 | 5.25E-07 | 0.003632198 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 221601_s_at | FAIM3 | Fas apoptotic inhibitory molecule 3 | -0.762837321 | 2.20E-06 | 0.005014178 |
| 1553369_at | FAM129C | family with sequence similarity 129, member C | -0.394493652 | 9.69E-06 | 0.006492519 |
| 230983_at | FAM129C | family with sequence similarity 129, member C | -0.60016683 | 6.69E-05 | 0.009195279 |
| 218510_x_at | FAM134B | family with sequence similarity 134, member B | -0.306567395 | 6.04E-05 | 0.008983607 |
| 218464_s_at | FAM222B | family with sequence similarity 222, member B | -0.299139145 | 6.88E-05 | 0.009195279 |
| 229289_at | FAM71E1 | family with sequence similarity 71, member E1 | 0.289799963 | 2.51E-05 | 0.007350583 |
| 212229_s_at | FBXO21 | F-box protein 21 | -0.429140649 | 8.40E-05 | 0.009786493 |
| 205310_at | FBXO46 | F-box protein 46 | -0.312595015 | 6.43E-05 | 0.009166579 |
| 235401_s_at | FCRLA | Fc receptor-like A | -1.074147189 | 1.58E-06 | 0.004572436 |
| 235372_at | FCRLA | Fc receptor-like A | -0.419492859 | 1.16E-05 | 0.006492519 |
| 207813_s_at | FDXR | ferredoxin reductase | 0.496086285 | 7.42E-11 | 4.06E-06 |
| 1556283_s_at | FGFR1OP2 | FGFR1 oncogene partner 2 | 0.851956179 | 6.53E-05 | 0.009173198 |
| 230389_at | FNBP1 | formin binding protein 1 | -0.410003097 | 5.84E-05 | 0.00881771 |
| 202723_s_at | FOXO1 | forkhead box O1 | -0.365678555 | 6.84E-05 | 0.009195279 |
| 223287_s_at | FOXP1 | forkhead box P1 | -0.493719018 | 4.04E-07 | 0.003632198 |
| 224838_at | FOXP1 | forkhead box P1 | -0.548101469 | 1.70E-06 | 0.004576131 |
| 224837_at | FOXP1 | forkhead box P1 | -0.525866739 | 2.70E-06 | 0.005447157 |
| 235444_at | FOXP1 | forkhead box P1 | -0.481176266 | 6.04E-06 | 0.006007512 |
| 1558996_at | FOXP1 | forkhead box P1 | -0.365210444 | 1.21E-05 | 0.006492519 |
| 229844_at | FOXP1 | forkhead box P1 | -0.346616329 | 6.76E-05 | 0.009195279 |
| 238076_at | GATAD2B | GATA zinc finger domain containing 2B | -0.293824643 | 7.47E-05 | 0.009405497 |
| 214711_at | GATC | glutamyl-tRNA(Gln) amidotransferase, subunit C | -0.385907352 | 7.56E-05 | 0.009464299 |
| 210565_at | GCGR | glucagon receptor | 0.347833617 | 1.47E-05 | 0.006523888 |
| 219508_at | GCNT3 | glucosaminyl (N-acetyl) transferase 3, mucin type | 0.298715293 | 2.63E-05 | 0.007381877 |
| 228173_at | GNAS | GNAS complex locus | -0.427370044 | 1.26E-05 | 0.006492519 |
| 214157_at | GNAS | GNAS complex locus | 0.572823182 | 0.00010718 | 0.01063539 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| | | | | 1 | 4 |
| 205042_at | GNE | glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase | -0.372407682 | 0.000116865 | 0.010991542 |
| 218873_at | GON4L | gon-4-like (C. elegans) | -0.300561751 | 8.47E-05 | 0.009786493 |
| 226429_at | GPALPP1 | GPALPP motifs containing 1 | -0.401653898 | 5.29E-05 | 0.008481109 |
| 212487_at | GPATCH8 | G patch domain containing 8 | -0.262831591 | 2.95E-05 | 0.007746832 |
| 214510_at | GPR20 | G protein-coupled receptor 20 | 0.271282071 | 2.96E-05 | 0.007746832 |
| 210411_s_at | GRIN2B | glutamate receptor, ionotropic, N-methyl D-aspartate 2B | 0.308973172 | 7.98E-05 | 0.009693645 |
| 243985_at | GTF2A2 | general transcription factor IIA, 2, 12kDa | -0.336767326 | 6.62E-05 | 0.009173198 |
| 229343_at | GTSE1 | G-2 and S-phase expressed 1 | 0.327675443 | 2.39E-05 | 0.007221094 |
| 220577_at | GVINP1 | GTPase, very large interferon inducible pseudogene 1 | -0.466920453 | 2.65E-05 | 0.007400143 |
| 207592_s_at | HCN2 | hyperpolarization activated cyclic nucleotide-gated potassium channel 2 | 0.363436439 | 6.01E-05 | 0.008983607 |
| 201209_at | HDAC1 | histone deacetylase 1 | -0.408992606 | 2.82E-05 | 0.007549101 |
| 218595_s_at | HEATR1 | HEAT repeat containing 1 | -0.439724396 | 3.21E-05 | 0.007775372 |
| 212642_s_at | HIVEP2 | human immunodeficiency virus type I enhancer binding protein 2 | -0.304928914 | 5.92E-05 | 0.008891725 |
| 212873_at | HMHA1 | histocompatibility (minor) HA-1 | -0.44566097 | 0.000115269 | 0.010922623 |
| 211930_at | HNRNPA3 | heterogeneous nuclear ribonucleoprotein A3 | -0.466998425 | 9.67E-05 | 0.010362258 |
| 209068_at | HNRNPDL | heterogeneous nuclear ribonucleoprotein D-like | -0.411190162 | 2.83E-05 | 0.007553933 |
| 213472_at | HNRNPH1 | heterogeneous nuclear ribonucleoprotein H1 (H) | -0.251006256 | 0.000111417 | 0.010789763 |
| 235603_at | HNRNPU | heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A) | -0.434544484 | 4.69E-05 | 0.008234897 |
| 205580_s_at | HRH1 | histamine receptor H1 | 0.204827226 | 2.42E-05 | 0.007221094 |
| 200064_at | HSP90AB1 | heat shock protein 90kDa alpha (cytosolic), class B member 1 | -0.526683057 | 3.82E-06 | 0.005447157 |
| 214359_s_at | HSP90AB1 | heat shock protein 90kDa alpha (cytosolic), class B member 1 | -0.58161724 | 6.98E-06 | 0.006063288 |
| 1557910_at | HSP90AB1 | heat shock protein 90kDa alpha (cytosolic), class B member 1 | -0.624542656 | 5.34E-05 | 0.008497204 |
| 211728_s_at | HYAL3 | hyaluronoglucosaminidase 3 | 0.275685263 | 4.61E-05 | 0.00818619 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| | | | | | 5 |
| 211693_at | IGH | immunoglobulin heavy locus | 0.330791865 | 6.18E-05 | 0.009050415 |
| 209374_s_at | IGHM | immunoglobulin heavy constant mu | -0.999878579 | 4.50E-06 | 0.005586248 |
| 212827_at | IGHM | immunoglobulin heavy constant mu | -1.326467867 | 9.48E-06 | 0.006492519 |
| 227344_at | IKZF1 | IKAROS family zinc finger 1 (Ikaros) | -0.306860047 | 2.61E-05 | 0.007381877 |
| 205039_s_at | IKZF1 | IKAROS family zinc finger 1 (Ikaros) | -0.416138023 | 6.32E-05 | 0.00914249 |
| 227030_at | IKZF3 | IKAROS family zinc finger 3 (Aiolos) | -0.519285046 | 2.18E-05 | 0.006985651 |
| 220663_at | IL1RAPL1 | interleukin 1 receptor accessory protein-like 1 | 0.378538632 | 2.93E-05 | 0.007746832 |
| 230966_at | IL4I1 | interleukin 4 induced 1 | 0.317913382 | 5.69E-05 | 0.008735693 |
| 204863_s_at | IL6ST | interleukin 6 signal transducer (gp130, oncostatin M receptor) | -0.556447548 | 2.63E-05 | 0.007381877 |
| 217805_at | ILF3 | interleukin enhancer binding factor 3, 90kDa | -0.543051101 | 8.06E-06 | 0.006439647 |
| 214705_at | INADL | InaD-like (Drosophila) | -0.493182156 | 0.000111427 | 0.010789763 |
| 202781_s_at | INPP5K | inositol polyphosphate-5-phosphatase K | 0.220926284 | 5.22E-05 | 0.008440745 |
| 217885_at | IPO9 | importin 9 | -0.379001483 | 2.21E-05 | 0.006985651 |
| 236767_at | IQCF2 | IQ motif containing F2 | 0.317469991 | 1.78E-05 | 0.006572198 |
| 223597_at | ITLN1 | intelectin 1 (galactofuranose binding) | 0.533194227 | 3.60E-05 | 0.007932185 |
| 203723_at | ITPKB | inositol-trisphosphate 3-kinase B | -0.362102492 | 1.07E-05 | 0.006492519 |
| 235213_at | ITPKB | inositol-trisphosphate 3-kinase B | -0.466134069 | 6.05E-05 | 0.008983607 |
| 240052_at | ITPR1 | inositol 1,4,5-trisphosphate receptor, type 1 | -0.617995157 | 5.44E-07 | 0.003632198 |
| 228074_at | ITPRIPL2 | inositol 1,4,5-trisphosphate receptor interacting protein-like 2 | 0.257475032 | 4.56E-05 | 0.008171695 |
| 212660_at | JADE2 | jade family PHD finger 2 | -0.56361616 | 7.73E-07 | 0.003642121 |
| 232279_at | JADE2 | jade family PHD finger 2 | -0.482609317 | 4.15E-06 | 0.005447157 |
| 209097_s_at | JAG1 | jagged 1 | 0.333511904 | 2.61E-05 | 0.007381877 |
| 221068_at | KANK2 | KN motif and ankyrin repeat domains 2 | 0.335290445 | 7.87E-05 | 0.009601968 |
| 208560_at | KCNA10 | potassium voltage-gated channel, shaker-related subfamily, member 10 | 0.302816681 | 5.36E-05 | 0.008497204 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 205968_at | KCNS3 | potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3 | 0.260977878 | 3.56E-05 | 0.007932185 |
| 214861_at | KDM4C | lysine (K)-specific demethylase 4C | -0.314236669 | 9.58E-05 | 0.010334733 |
| 223161_at | KIAA1147 | KIAA1147 | -0.356506618 | 6.97E-05 | 0.009195279 |
| 235956_at | KIAA1377 | KIAA1377 | -0.294197572 | 3.49E-05 | 0.007932185 |
| 210504_at | KLF1 | Kruppel-like factor 1 (erythroid) | 0.692994359 | 9.09E-05 | 0.010143703 |
| 209254_at | KLHDC10 | kelch domain containing 10 | 0.380661547 | 4.77E-05 | 0.008244777 |
| 221221_s_at | KLHL3 | kelch-like family member 3 | -0.380996345 | 3.01E-05 | 0.007762527 |
| 219692_at | KREMEN2 | kringle containing transmembrane protein 2 | 0.213821988 | 8.47E-05 | 0.009786493 |
| 222427_s_at | LARS | leucyl-tRNA synthetase | -0.458020559 | 1.50E-05 | 0.006523888 |
| 222428_s_at | LARS | leucyl-tRNA synthetase | -0.484242977 | 4.82E-05 | 0.008292277 |
| 57082_at | LDLRAP1 | low density lipoprotein receptor adaptor protein 1 | -0.485918345 | 3.39E-06 | 0.005447157 |
| 221790_s_at | LDLRAP1 | low density lipoprotein receptor adaptor protein 1 | -0.400002032 | 6.74E-05 | 0.009195279 |
| 221558_s_at | LEF1 | lymphoid enhancer-binding factor 1 | -0.705786773 | 3.60E-05 | 0.007932185 |
| 231760_at | LINC00029 | long intergenic non-protein coding RNA 29 | 0.255109118 | 8.21E-05 | 0.009761164 |
| 230245_s_at | LINC00926 | long intergenic non-protein coding RNA 926 | -0.95766674 | 2.37E-05 | 0.007221094 |
| 227933_at | LINGO1 | leucine rich repeat and Ig domain containing 1 | 0.282439379 | 1.40E-05 | 0.006492519 |
| 243703_x_at | LIPE-AS1 | LIPE antisense RNA 1 | 0.196463345 | 4.85E-05 | 0.008292277 |
| 243523_at | LOC100128644 | LMNE6487 | 0.318268467 | 7.00E-05 | 0.009195279 |
| 1558569_at | LOC100131541 | uncharacterized LOC100131541 | -0.514351109 | 5.78E-05 | 0.008795965 |
| 237977_at | LOC100996349 | testis expressed 264 pseudogene | 0.296979507 | 2.19E-05 | 0.006985651 |
| 228808_s_at | LOXL2 | lysyl oxidase-like 2 | 0.304366291 | 4.55E-05 | 0.008171695 |
| 230252_at | LPAR5 | lysophosphatidic acid receptor 5 | -0.415732602 | 4.66E-05 | 0.008234897 |
| 219922_s_at | LTBP3 | latent transforming growth factor beta binding protein 3 | -0.513151377 | 5.48E-06 | 0.00592514 |
| 206480_at | LTC4S | leukotriene C4 synthase | 0.217934899 | 0.000101852 | 0.010514226 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 1557067_s_at | LUC7L | LUC7-like (S. cerevisiae) | -0.360993579 | 4.93E-05 | 0.00833877 |
| 231840_x_at | LYRM7 | LYR motif containing 7 | -0.398090127 | 0.000103219 | 0.010514226 |
| 235457_at | MAML2 | mastermind-like 2 (Drosophila) | -0.415702498 | 7.91E-05 | 0.009627636 |
| 238993_at | MATR3 | matrin 3 | 0.30822919 | 1.30E-05 | 0.006492519 |
| 205655_at | MDM4 | MDM4, p53 regulator | -0.544074949 | 2.79E-05 | 0.007549101 |
| 236814_at | MDM4 | MDM4, p53 regulator | -0.475153913 | 0.000110218 | 0.010780225 |
| 212693_at | MDN1 | MDN1, midasin homolog (yeast) | -0.429456993 | 8.85E-06 | 0.006492519 |
| 1569484_s_at | MDN1 | MDN1, midasin homolog (yeast) | -0.435067498 | 9.46E-05 | 0.010297887 |
| 203496_s_at | MED1 | mediator complex subunit 1 | -0.282987039 | 3.03E-05 | 0.007762527 |
| 225452_at | MED1 | mediator complex subunit 1 | -0.364159712 | 4.42E-05 | 0.008171695 |
| 203497_at | MED1 | mediator complex subunit 1 | -0.469369383 | 0.000101531 | 0.010514226 |
| 226744_at | METTL16 | methyltransferase like 16 | -0.422281378 | 1.34E-05 | 0.006492519 |
| 222042_x_at | MEX3D | mex-3 RNA binding family member D | 0.285693523 | 8.05E-05 | 0.009694254 |
| 235409_at | MGA | MGA, MAX dimerization protein | -0.441315705 | 3.83E-05 | 0.007932185 |
| 231283_at | MGAT4A | mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A | -0.296731093 | 0.000114317 | 0.010851164 |
| 212098_at | MGAT5 | mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase | -0.286738537 | 6.89E-05 | 0.009195279 |
| 1569652_at | MLLT3 | myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3 | -0.496122852 | 8.58E-05 | 0.00983175 |
| 202167_s_at | MMS19 | MMS19 nucleotide excision repair homolog (S. cerevisiae) | -0.327500445 | 9.12E-06 | 0.006492519 |
| 203956_at | MORC2 | MORC family CW-type zinc finger 2 | -0.39617249 | 1.33E-05 | 0.006492519 |
| 225041_at | MPHOSPH8 | M-phase phosphoprotein 8 | -0.519073965 | 8.00E-05 | 0.009694254 |
| 243801_x_at | MRPL30 | mitochondrial ribosomal protein L30 | -0.430521081 | 7.35E-05 | 0.009350761 |
| 217418_x_at | MS4A1 | membrane-spanning 4-domains, subfamily A, member 1 | -1.112188072 | 5.98E-06 | 0.006007512 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 210356_x_at | MS4A1 | membrane-spanning 4-domains, subfamily A, member 1 | -1.092374741 | 1.14E-05 | 0.006492519 |
| 231418_at | MS4A1 | membrane-spanning 4-domains, subfamily A, member 1 | -0.800582465 | 1.90E-05 | 0.006781334 |
| 228592_at | MS4A1 | membrane-spanning 4-domains, subfamily A, member 1 | -1.423438894 | 6.41E-05 | 0.009166579 |
| 225240_s_at | MSI2 | musashi RNA-binding protein 2 | -0.533898671 | 4.58E-05 | 0.008186195 |
| 202431_s_at | MYC | v-myc avian myelocytomatosis viral oncogene homolog | -0.592490345 | 4.92E-07 | 0.003632198 |
| 201959_s_at | MYCBP2 | MYC binding protein 2, E3 ubiquitin protein ligase | -0.486500503 | 3.80E-06 | 0.005447157 |
| 201960_s_at | MYCBP2 | MYC binding protein 2, E3 ubiquitin protein ligase | -0.436992278 | 4.41E-05 | 0.008171695 |
| 213613_s_at | NADK | NAD kinase | 0.274914967 | 3.58E-05 | 0.007932185 |
| 238722_x_at | NAPEPLD | N-acyl phosphatidylethanolamine phospholipase D | -0.576017953 | 3.42E-06 | 0.005447157 |
| 212854_x_at | NBPF1 | neuroblastoma breakpoint family, member 1 | -0.353971714 | 9.94E-05 | 0.010468604 |
| 242191_at | NBPF10 | neuroblastoma breakpoint family, member 10 | -0.591032863 | 9.05E-06 | 0.006492519 |
| 211105_s_at | NFATC1 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | -0.347685471 | 4.24E-06 | 0.005447157 |
| 225876_at | NIPAL3 | NIPA-like domain containing 3 | -0.487868543 | 9.48E-06 | 0.006492519 |
| 234762_x_at | NLN | neurolysin (metallopeptidase M3 family) | -0.52211926 | 7.81E-05 | 0.009573853 |
| 231798_at | NOG | noggin | -0.590006121 | 2.25E-05 | 0.007023341 |
| 211951_at | NOLC1 | nucleolar and coiled-body phosphoprotein 1 | -0.37959983 | 9.47E-05 | 0.010297887 |
| 229220_x_at | NOM1 | nucleolar protein with MIF4G domain 1 | -0.362244923 | 8.39E-05 | 0.009786493 |
| 214427_at | NOP2 | NOP2 nucleolar protein | -0.339299058 | 3.86E-05 | 0.007932185 |
| 210808_s_at | NOX1 | NADPH oxidase 1 | 0.250506077 | 5.14E-05 | 0.008414453 |
| 204538_x_at | NPIPA1 | nuclear pore complex interacting protein family, member A1 | -0.38699399 | 2.74E-05 | 0.007481573 |
| 215921_at | NPIPB3 | nuclear pore complex interacting protein family, member B3 | -0.431076797 | 0.000103355 | 0.010514226 |
| 229122_x_at | NPRL3 | nitrogen permease regulator-like 3 (S. cerevisiae) | 0.306761196 | 8.63E-05 | 0.009846679 |
| 209261_s_at | NR2F6 | nuclear receptor subfamily 2, group F, member 6 | 0.229121513 | 9.75E-05 | 0.010413787 |
| 226499_at | NRARP | NOTCH-regulated ankyrin repeat protein | 0.28415589 | 8.45E-05 | 0.009786493 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 203802_x_at | NSUN5 | NOP2/Sun domain family, member 5 | -0.313367587 | 6.04E-06 | 0.006007512 |
| 213670_x_at | NSUN5P1 | NOP2/Sun domain family, member 5 pseudogene 1 | -0.38727319 | 3.11E-05 | 0.007762527 |
| 234496_x_at | NYX | nyctalopin | 0.360611808 | 8.39E-05 | 0.009786493 |
| 239748_x_at | OCIAD1 | OCIA domain containing 1 | -0.401248072 | 9.27E-05 | 0.010202143 |
| 1565065_at | OFCC1 | orofacial cleft 1 candidate 1 | 0.243983021 | 0.000111173 | 0.010789763 |
| 241751_at | OFD1 | oral-facial-digital syndrome 1 | -0.608050623 | 3.03E-05 | 0.007762527 |
| 225106_s_at | OGFOD1 | 2-oxoglutarate and iron-dependent oxygenase domain containing 1 | -0.393230181 | 2.20E-05 | 0.006985651 |
| 1569617_at | OSBP2 | oxysterol binding protein 2 | 0.288132635 | 6.47E-05 | 0.009166579 |
| 219073_s_at | OSBPL10 | oxysterol binding protein-like 10 | -0.813152852 | 4.27E-05 | 0.008151598 |
| 221209_s_at | OTOR | otoraplin | 0.314067788 | 0.000106984 | 0.010635394 |
| 227686_at | OXNAD1 | oxidoreductase NAD-binding domain containing 1 | -0.440066938 | 1.12E-05 | 0.006492519 |
| 210448_s_at | P2RX5 | purinergic receptor P2X, ligand-gated ion channel, 5 | -0.72013783 | 1.57E-06 | 0.004572436 |
| 232683_s_at | PARP6 | poly (ADP-ribose) polymerase family, member 6 | -0.312101792 | 5.66E-05 | 0.008735693 |
| 221969_at | PAX5 | paired box 5 | -0.952108039 | 3.84E-06 | 0.005447157 |
| 235482_at | PCBP1-AS1 | PCBP1 antisense RNA 1 | -0.482781755 | 2.73E-05 | 0.007481573 |
| 222380_s_at | PDCD6 | programmed cell death 6 | -0.393322931 | 6.45E-05 | 0.009166579 |
| 1552343_s_at | PDE7A | phosphodiesterase 7A | -0.346973621 | 7.63E-05 | 0.009481454 |
| 223619_x_at | PECR | peroxisomal trans-2-enoyl-CoA reductase | -0.339891001 | 9.30E-05 | 0.010211571 |
| 209346_s_at | PI4K2A | phosphatidylinositol 4-kinase type 2 alpha | 0.261239358 | 6.75E-05 | 0.009195279 |
| 209018_s_at | PINK1 | PTEN induced putative kinase 1 | 0.364308832 | 8.45E-05 | 0.009786493 |
| 227419_x_at | PLAC9 | placenta-specific 9 | 0.261871268 | 7.08E-05 | 0.009212663 |
| 1557126_a_at | PLD1 | phospholipase D1, phosphatidylcholine-specific | 0.233744872 | 3.12E-05 | 0.007762527 |
| 226122_at | PLEKHG1 | pleckstrin homology domain containing, family G (with RhoGef domain) member 1 | -0.328136583 | 1.25E-05 | 0.006492519 |
| 216843_x_at | PMS2P1 | postmeiotic segregation increased 2 pseudogene 1 | -0.37162667 | 2.49E-05 | 0.007350583 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 217485_x_at | PMS2P1 | postmeiotic segregation increased 2 pseudogene 1 | -0.283501796 | 3.40E-05 | 0.007885737 |
| 212177_at | PNISR | PNN-interacting serine/arginine-rich protein | -0.475076915 | 4.61E-05 | 0.008186195 |
| 225507_at | PNISR | PNN-interacting serine/arginine-rich protein | -0.341568865 | 6.97E-05 | 0.009195279 |
| 205267_at | POU2AF1 | POU class 2 associating factor 1 | -1.04247299 | 3.89E-05 | 0.007932185 |
| 37152_at | PPARD | peroxisome proliferator-activated receptor delta | -0.297888757 | 6.96E-05 | 0.009195279 |
| 226773_at | PPM1K | protein phosphatase, Mg2+/Mn2+ dependent, 1K | -0.511916974 | 2.08E-05 | 0.006810658 |
| 235061_at | PPM1K | protein phosphatase, Mg2+/Mn2+ dependent, 1K | -0.726983464 | 0.000106066 | 0.01062351 |
| 229001_at | PPP1R3E | protein phosphatase 1, regulatory subunit 3E | -0.432051845 | 3.55E-06 | 0.005447157 |
| 227412_at | PPP1R3E | protein phosphatase 1, regulatory subunit 3E | -0.372991128 | 1.21E-05 | 0.006492519 |
| 213093_at | PRKCA | protein kinase C, alpha | -0.512082012 | 2.73E-05 | 0.007481573 |
| 212068_s_at | PRRC2B | proline-rich coiled-coil 2B | -0.276950594 | 4.16E-06 | 0.005447157 |
| 214055_x_at | PRRC2C | proline-rich coiled-coil 2C | -0.430957432 | 6.26E-05 | 0.009096416 |
| 211948_x_at | PRRC2C | proline-rich coiled-coil 2C | -0.400712638 | 9.18E-05 | 0.010159282 |
| 226491_x_at | PTBP1 | polypyrimidine tract binding protein 1 | 0.210336454 | 9.26E-05 | 0.010202143 |
| 238754_at | PTCH1 | patched 1 | 0.38726248 | 2.61E-05 | 0.007381877 |
| 234000_s_at | PTPLAD1 | protein tyrosine phosphatase-like A domain containing 1 | -0.496474236 | 7.83E-05 | 0.009573853 |
| 239526_x_at | PTPN1 | protein tyrosine phosphatase, non-receptor type 1 | 0.262946747 | 1.04E-05 | 0.006492519 |
| 201164_s_at | PUM1 | pumilio RNA-binding family member 1 | -0.321931161 | 0.000117002 | 0.010991542 |
| 218949_s_at | QRSL1 | glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1 | -0.442270284 | 7.09E-05 | 0.009212663 |
| 1556122_at | RAB11B-AS1 | RAB11B antisense RNA 1 | 0.23101354 | 4.50E-05 | 0.008171695 |
| 229072_at | RAB30 | RAB30, member RAS oncogene family | -0.665492593 | 4.23E-06 | 0.005447157 |
| 228390_at | RAB30 | RAB30, member RAS oncogene family | -0.725204554 | 3.60E-05 | 0.007932185 |
| 74694_s_at | RABEP2 | rabaptin, RAB GTPase binding effector protein 2 | -0.587874223 | 7.38E-05 | 0.009355008 |
| 226090_x_at | RABL3 | RAB, member of RAS oncogene family-like 3 | -0.359080371 | 8.05E-06 | 0.006439647 |
| 202482_x_at | RANBP1 | RAN binding protein 1 | 0.333432015 | 3.94E-05 | 0.00793218 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| | | | | | 5 |
| 208206_s_at | RASGRP2 | RAS guanyl releasing protein 2 (calcium and DAG-regulated) | -0.410068928 | 1.07E-05 | 0.006492519 |
| 205801_s_at | RASGRP3 | RAS guanyl releasing protein 3 (calcium and DAG-regulated) | -0.469693555 | 3.51E-05 | 0.007932185 |
| 205178_s_at | RBBP6 | retinoblastoma binding protein 6 | -0.379184711 | 8.27E-06 | 0.006439647 |
| 212783_at | RBBP6 | retinoblastoma binding protein 6 | -0.421076872 | 1.44E-05 | 0.006492519 |
| 216153_x_at | RECK | reversion-inducing-cysteine-rich protein with kazal motifs | -0.238139538 | 9.99E-05 | 0.010500921 |
| 236690_at | RHBDD1 | rhomboid domain containing 1 | 0.314583324 | 7.76E-05 | 0.009573853 |
| 220510_at | RHBG | Rh family, B glycoprotein (gene/pseudogene) | 0.295950502 | 6.62E-05 | 0.009173198 |
| 210430_x_at | RHD | Rh blood group, D antigen | 0.408141193 | 2.34E-05 | 0.007193001 |
| 236293_at | RHOH | ras homolog family member H | -0.639756696 | 4.48E-05 | 0.008171695 |
| 205211_s_at | RIN1 | Ras and Rab interactor 1 | 0.343377143 | 5.20E-06 | 0.00592514 |
| 207735_at | RNF125 | ring finger protein 125, E3 ubiquitin protein ligase | -0.353981086 | 7.14E-05 | 0.009212663 |
| 238055_at | RP11-35G9.3 | uncharacterized LOC100505549 | -0.382736444 | 3.05E-05 | 0.007762527 |
| 1560337_at | RP11-579E24.1 | uncharacterized LOC286184 | 0.255707843 | 9.84E-05 | 0.010437856 |
| 200003_s_at | RPL28 | ribosomal protein L28 | -0.491367012 | 7.82E-05 | 0.009573853 |
| 226078_at | RPUSD1 | RNA pseudouridylate synthase domain containing 1 | 0.243727986 | 9.79E-05 | 0.010434702 |
| 213495_s_at | RRBP1 | ribosome binding protein 1 | 0.251436629 | 8.19E-05 | 0.009761164 |
| 209773_s_at | RRM2 | ribonucleotide reductase M2 | 0.302760075 | 5.13E-05 | 0.008414453 |
| 212846_at | RRP1B | ribosomal RNA processing 1B | -0.587984837 | 3.36E-05 | 0.007849706 |
| 222789_at | RSBN1 | round spermatid basic protein 1 | -0.431389282 | 2.93E-06 | 0.005447157 |
| 230700_at | RTN4RL1 | reticulon 4 receptor-like 1 | 0.368751512 | 3.70E-05 | 0.007932185 |
| 218677_at | S100A14 | S100 calcium binding protein A14 | 0.302917519 | 1.41E-05 | 0.006492519 |
| 203408_s_at | SATB1 | SATB homeobox 1 | -0.425846308 | 3.08E-05 | 0.007762527 |
| 232992_at | SAYSD1 | SAYSVFN motif domain containing 1 | 0.251863532 | 1.92E-05 | 0.006791288 |
| 1569225_a_at | SCML4 | sex comb on midleg-like 4 (Drosophila) | -0.653984768 | 3.59E-05 | 0.007932185 |
| 224029_x_at | SCN11A | sodium channel, voltage-gated, | -0.151498324 | 0.00010473 | 0.01060070 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| | | type XI, alpha subunit | | 4 | 3 |
| 212314_at | SEL1L3 | sel-1 suppressor of lin-12-like 3 (C. elegans) | -0.682941456 | 6.04E-05 | 0.008983607 |
| 235684_s_at | SESN3 | sestrin 3 | 0.649850264 | 8.38E-05 | 0.009786493 |
| 221806_s_at | SETD5 | SET domain containing 5 | -0.334765544 | 0.000105102 | 0.010602277 |
| 208313_s_at | SF1 | splicing factor 1 | -0.393656849 | 3.79E-05 | 0.007932185 |
| 215454_x_at | SFTPC | surfactant protein C | 0.356717469 | 0.000106959 | 0.010635394 |
| 219734_at | SIDT1 | SID1 transmembrane family, member 1 | -0.485749263 | 0.000104892 | 0.010600703 |
| 52940_at | SIGIRR | single immunoglobulin and toll-interleukin 1 receptor (TIR) domain | -0.395139839 | 3.37E-05 | 0.007849706 |
| 1563498_s_at | SLC25A45 | solute carrier family 25, member 45 | -0.424836621 | 2.01E-05 | 0.006791288 |
| 207560_at | SLC28A1 | solute carrier family 28 (concentrative nucleoside transporter), member 1 | 0.316246373 | 5.98E-07 | 0.003632198 |
| 218237_s_at | SLC38A1 | solute carrier family 38, member 1 | -0.637173145 | 1.20E-05 | 0.006492519 |
| 222935_x_at | SLC39A8 | solute carrier family 39 (zinc transporter), member 8 | 0.250769992 | 0.00010346 | 0.010514226 |
| 234291_s_at | SLC6A20 | solute carrier family 6 (proline IMINO transporter), member 20 | 0.374834393 | 1.71E-05 | 0.006523888 |
| 206565_x_at | SMA4 | glucuronidase, beta pseudogene | -0.398084569 | 7.43E-05 | 0.009382524 |
| 214728_x_at | SMARCA4 | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 | -0.238489293 | 0.000105391 | 0.010611894 |
| 215383_x_at | SPG21 | spastic paraplegia 21 (autosomal recessive, Mast syndrome) | -0.247742114 | 6.89E-05 | 0.009195279 |
| 205861_at | SPIB | Spi-B transcription factor (Spi-1/PU.1 related) | -0.471726559 | 3.27E-05 | 0.007834974 |
| 202524_s_at | SPOCK2 | sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2 | -0.522745927 | 8.04E-05 | 0.009694254 |
| 212071_s_at | SPTBN1 | spectrin, beta, non-erythrocytic 1 | -0.600229037 | 4.54E-05 | 0.008171695 |
| 224145_s_at | SPTBN4 | spectrin, beta, non-erythrocytic 4 | 0.327763039 | 7.26E-05 | 0.009255079 |
| 222047_s_at | SRRT | serrate RNA effector molecule homolog (Arabidopsis) | -0.396830354 | 8.11E-05 | 0.009698499 |
| 201680_x_at | SRRT | serrate RNA effector molecule homolog (Arabidopsis) | -0.346178403 | 9.52E-05 | 0.010312924 |
| 213649_at | SRSF7 | serine/arginine-rich splicing factor 7 | -0.561980049 | 3.70E-05 | 0.007932185 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 211670_x_at | SSX3 | synovial sarcoma, X breakpoint 3 | 0.297606746 | 6.99E-05 | 0.009195279 |
| 220059_at | STAP1 | signal transducing adaptor family member 1 | -0.760330756 | 2.95E-05 | 0.007746832 |
| 233252_s_at | STRBP | spermatid perinuclear RNA binding protein | -0.625885419 | 4.72E-06 | 0.005730929 |
| 233251_at | STRBP | spermatid perinuclear RNA binding protein | -0.427149645 | 1.14E-05 | 0.006492519 |
| 229513_at | STRBP | spermatid perinuclear RNA binding protein | -0.87095208 | 1.98E-05 | 0.006791288 |
| 223548_at | SWT1 | SWT1 RNA endoribonuclease homolog (S. cerevisiae) | 0.277938209 | 8.30E-05 | 0.009786493 |
| 222715_s_at | SYNRG | synergin, gamma | -0.530678994 | 3.71E-05 | 0.007932185 |
| 64418_at | SYNRG | synergin, gamma | -0.417960808 | 3.72E-05 | 0.007932185 |
| 201259_s_at | SYPL1 | synaptophysin-like 1 | -0.420077785 | 0.000101727 | 0.010514226 |
| 1562255_at | SYTL3 | synaptotagmin-like 3 | -0.494659965 | 1.74E-05 | 0.006566687 |
| 237091_at | TBC1D9 | TBC1 domain family, member 9 (with GRAM domain) | 0.332708125 | 9.65E-05 | 0.010362258 |
| 220634_at | TBX4 | T-box 4 | 0.319121387 | 6.70E-05 | 0.009195279 |
| 212386_at | TCF4 | transcription factor 4 | -0.594537057 | 4.74E-05 | 0.008244777 |
| 205254_x_at | TCF7 | transcription factor 7 (T-cell specific, HMG-box) | -0.498578743 | 2.02E-06 | 0.004804804 |
| 205255_x_at | TCF7 | transcription factor 7 (T-cell specific, HMG-box) | -0.662699679 | 3.23E-05 | 0.007782739 |
| 39318_at | TCL1A | T-cell leukemia/lymphoma 1A | -0.977364696 | 4.52E-05 | 0.008171695 |
| 209995_s_at | TCL1A | T-cell leukemia/lymphoma 1A | -0.912314963 | 0.000103175 | 0.010514226 |
| 1553007_a_at | TENM1 | teneurin transmembrane protein 1 | 0.284576884 | 5.35E-05 | 0.008497204 |
| 207883_s_at | TFR2 | transferrin receptor 2 | 0.299146671 | 8.56E-05 | 0.00983175 |
| 217567_at | TGM4 | transglutaminase 4 | 0.314926017 | 9.38E-05 | 0.010253846 |
| 222122_s_at | THOC2 | THO complex 2 | -0.428635026 | 4.07E-05 | 0.007970846 |
| 215168_at | TIMM17A | translocase of inner mitochondrial membrane 17 homolog A (yeast) | 0.175341251 | 2.03E-05 | 0.006791288 |
| 222904_s_at | TMC5 | transmembrane channel-like 5 | 0.246635076 | 8.30E-05 | 0.009786493 |
| 227353_at | TMC8 | transmembrane channel-like 8 | -0.466475369 | 0.000107871 | 0.010665225 |
| 224493_x_at | TMEM241 | transmembrane protein 241 | -0.291964579 | 4.42E-05 | 0.008171695 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 214833_at | TMEM63A | transmembrane protein 63A | -0.469887613 | 6.77E-06 | 0.006063288 |
| 231775_at | TNFRSF10A | tumor necrosis factor receptor superfamily, member 10a | -0.521556159 | 4.47E-05 | 0.008171695 |
| 209225_x_at | TNPO1 | transportin 1 | -0.302582222 | 0.000111986 | 0.010789763 |
| 221829_s_at | TNPO1 | transportin 1 | -0.395480058 | 0.000115925 | 0.01096576 |
| 238468_at | TNRC6B | trinucleotide repeat containing 6B | -0.378292168 | 1.66E-05 | 0.006523888 |
| 230779_at | TNRC6B | trinucleotide repeat containing 6B | -0.424240404 | 4.00E-05 | 0.007932185 |
| 222820_at | TNRC6C | trinucleotide repeat containing 6C | -0.486061295 | 6.90E-05 | 0.009195279 |
| 242364_x_at | TNRC6C-AS1 | TNRC6C antisense RNA 1 | -0.354561084 | 2.34E-05 | 0.007193001 |
| 215275_at | TRAF3IP3 | TRAF3 interacting protein 3 | -0.423073431 | 4.83E-05 | 0.008292277 |
| 240265_at | TRAF3IP3 | TRAF3 interacting protein 3 | -0.618139183 | 6.12E-05 | 0.009021899 |
| 205804_s_at | TRAF3IP3 | TRAF3 interacting protein 3 | -0.404212455 | 0.000104381 | 0.010588156 |
| 200990_at | TRIM28 | tripartite motif containing 28 | -0.367754296 | 3.69E-05 | 0.007932185 |
| 217759_at | TRIM44 | tripartite motif containing 44 | -0.429987772 | 1.52E-05 | 0.006523888 |
| 217760_at | TRIM44 | tripartite motif containing 44 | -0.284441617 | 6.91E-05 | 0.009195279 |
| 219405_at | TRIM68 | tripartite motif containing 68 | -0.337082154 | 3.94E-05 | 0.007932185 |
| 1554250_s_at | TRIM73 | tripartite motif containing 73 | -0.620773737 | 4.96E-05 | 0.00833877 |
| 232489_at | TRMT13 | tRNA methyltransferase 13 homolog (S. cerevisiae) | -0.63902291 | 3.95E-05 | 0.007932185 |
| 233617_at | TSPY26P | testis specific protein, Y-linked 26, pseudogene | 0.264522948 | 5.54E-05 | 0.008663178 |
| 232323_s_at | TTC17 | tetratricopeptide repeat domain 17 | -0.440262054 | 6.51E-06 | 0.006034078 |
| 210389_x_at | TUBD1 | tubulin, delta 1 | -0.28335485 | 5.83E-05 | 0.00881771 |
| 212337_at | TUG1 | taurine up-regulated 1 (non-protein coding) | -0.333775066 | 5.38E-05 | 0.008505056 |
| 244199_at | TWF1 | twinfilin actin-binding protein 1 | 0.366021401 | 0.000101507 | 0.010514226 |
| 210065_s_at | UPK1B | uroplakin 1B | 0.300377871 | 1.64E-05 | 0.006523888 |
| 208723_at | USP11 | ubiquitin specific peptidase 11 | -0.317653056 | 3.76E-05 | 0.007932185 |
| 226477_at | VPRBP | Vpr (HIV-1) binding protein | -0.389907839 | 3.51E-05 | 0.007932185 |

| ID | Symbol | Name | Log(Fold-Change) | P.Value | adj.P.Val |
|---|---|---|---|---|---|
| 1560059_at | VPS37C | vacuolar protein sorting 37 homolog C (S. cerevisiae) | 0.322119039 | 9.13E-05 | 0.010159282 |
| 242211_x_at | WDR90 | WD repeat domain 90 | 0.294756164 | 9.32E-05 | 0.010212973 |
| 206698_at | XK | X-linked Kx blood group (McLeod syndrome) | 0.982140408 | 0.000116359 | 0.010972383 |
| 221939_at | YIPF2 | Yip1 domain family, member 2 | 0.255451089 | 5.72E-05 | 0.008741096 |
| 219075_at | YIPF2 | Yip1 domain family, member 2 | 0.344417961 | 9.47E-05 | 0.010297887 |
| 212340_at | YIPF6 | Yip1 domain family, member 6 | 0.378058588 | 1.59E-06 | 0.004572436 |
| 1557065_at | YLPM1 | YLP motif containing 1 | -0.455025125 | 1.17E-05 | 0.006492519 |
| 212787_at | YLPM1 | YLP motif containing 1 | -0.390242172 | 0.000108486 | 0.010704185 |
| 235308_at | ZBTB20 | zinc finger and BTB domain containing 20 | -0.511911858 | 4.54E-07 | 0.003632198 |
| 205383_s_at | ZBTB20 | zinc finger and BTB domain containing 20 | -0.418530764 | 1.82E-05 | 0.006619451 |
| 222357_at | ZBTB20 | zinc finger and BTB domain containing 20 | -0.544804149 | 4.29E-05 | 0.008151598 |
| 213051_at | ZC3HAV1 | zinc finger CCCH-type, antiviral 1 | -0.438161238 | 0.000113977 | 0.010851164 |
| 230332_at | ZCCHC7 | zinc finger, CCHC domain containing 7 | -0.592734231 | 1.37E-05 | 0.006492519 |
| 218077_s_at | ZDHHC3 | zinc finger, DHHC-type containing 3 | 0.26795685 | 3.05E-05 | 0.007762527 |
| 202456_s_at | ZER1 | zyg-11 related, cell cycle regulator | 0.406221836 | 3.20E-05 | 0.007775372 |
| 214142_at | ZG16 | zymogen granule protein 16 | 0.335494741 | 2.78E-05 | 0.007549101 |
| 209049_s_at | ZMYND8 | zinc finger, MYND-type containing 8 | -0.376690366 | 4.32E-05 | 0.008151598 |
| 239231_at | ZNF101 | zinc finger protein 101 | -0.531112138 | 4.19E-05 | 0.008100378 |
| 217403_s_at | ZNF227 | zinc finger protein 227 | -0.326091382 | 7.17E-05 | 0.009224321 |
| 239145_at | ZNF414 | zinc finger protein 414 | 0.40571468 | 3.76E-05 | 0.007932185 |
| 217627_at | ZNF573 | zinc finger protein 573 | -0.642804494 | 1.13E-05 | 0.006492519 |
| 223366_at | ZNF704 | zinc finger protein 704 | 0.267992843 | 1.97E-05 | 0.006791288 |
| 227734_s_at | ZNHIT2 | zinc finger, HIT-type containing 2 | 0.285455678 | 0.000107429 | 0.010640774 |
| | | | | | |